AO 120 (Rev. 2/99)

TO: **Mail Stop 8** **Director of the U.S. Patent & Trademark Office** **P.O. Box 1450** **Alexandria, VA 22313-1450**	**REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK**

In Compliance with 35 § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been filed in the U.S. District Court Northern District, San Francisco on the following X Patents or ☐ Trademarks:

DOCKET NO.	DATE FILED	U.S. DISTRICT COURT
CV 10-00675 JSW	02/17/2010	Northern District, San Francisco

PLAINTIFF	DEFENDANT
GLAXO GROUP LIMITED	GENENTECH INC

	PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1	6,331,415		(See Complaint)
2			
3			
4			
5			

In the above—entitled case, the following patent(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1		
2		
3		
4		
5		

In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE
Richard W. Wieking	William Noble	February 18, 2010

Copy 1—Upon initiation of action, mail this copy to Commissioner **Copy 3—Upon termination of action, mail this copy to Commissioner**
Copy 2—Upon filing document adding patent(s), mail this copy to Commissioner **Copy 4—Case file copy**

GSK'S OFATUMUMAB (ARZERRA™)

27. Ofatumumab (Arzerra™) is a new, human monoclonal antibody which targets the CD20 antigen, a naturally occurring protein present on B-lymphocytes, which is believed to be involved in the mediation of lymphoproliferative and autoimmune diseases.

28. Genmab A/S originally developed ofatumumab. In December 2006, GSK and Genmab A/S entered into an agreement to co-develop ofatumumab for therapeutic use. Under the terms of its agreements with Genmab, GSK has the exclusive right to make, use, import, offer to sell, and sell ofatumumab (Arzerra™) in the United States.

29. Pursuant to a contract with GSK, Lonza Biologics plc currently manufactures Arzerra™ in the United Kingdom for commercial sale by GSK in the United States. In addition, copies of the working cell bank used to produce Arzerra™ are maintained by Lonza Biologics, Inc. in Portsmouth, New Hampshire. On information and belief, Lonza Biologics plc and Lonza Biologics, Inc. (collectively "Lonza") may have received from Genentech certain rights or covenants not to sue relating to the Cabilly II patent pursuant to the 2001 Settlement Agreement between Celltech and Genentech. The scope of those rights, however, is confidential and unknown to GSK, despite reasonable efforts to ascertain what, if any, rights Lonza may have. Therefore, Genentech has affirmed through its conduct and agreement with Lonza that permission is needed from Genentech for Lonza to manufacture recombinant antibody products.

30. On October 26, 2009, GSK received accelerated approval from the U.S. Food and Drug Administration ("FDA") to market Arzerra™ in the United States for the treatment of patients whose chronic lymphocytic leukemia ("CLL") is refractory to previous therapies (fludarabine and alemtuzumab). Following that approval, GSK has begun marketing and selling Arzerra™ in the United States, doctors have begun prescribing Arzerra™, and patients suffering from refractory CLL have begun taking Arzerra™ to treat their CLL.

GSK'S DISPUTE WITH GENENTECH REGARDING CABILLY II PATENT

31. Through its statements and actions, Genentech has made clear to the biopharmaceutical industry generally and to GSK that it contends that the claims of the Cabilly II patent effectively preclude others from commercially manufacturing recombinant monoclonal

antibodies without Genentech's permission. In 2002, after the Cabilly II patent issued, Sean Johnston, then Genentech's Vice President of Intellectual Property and now Genentech's Senior Vice President and General Counsel said:

> "The recently issued patent **broadly covers** the co-expression of immunoglobulin heavy and light chain genes in a single host cell . . . We do not believe that the claims are limited by type of antibody (murine, humanized [90% human sequence], or human) or by host cell type."

("Genentech Awarded Critical Antibody Patent," *Nature Biotechnology*, vol. 20, p. 108 (Feb. 2002) (emphasis added).

32. According to Defendants, the manufacturing methods claimed in the Cabilly II patent are "the backbone of recombinant antibody production in the biotech industry." (*Centocor, Inc. v. Genentech, Inc.*, Case No. 2:08-cv-03573-MRP-CT (C.D. Cal.), 3/24/09 Opening Brief of Claim Construction).

33. Genentech has asserted the Cabilly II patent in litigation against other manufacturers of recombinant monoclonal antibodies, including MedImmune, Inc. ("MedImmune") and Centocor Ortho Biotech Inc. ("Centocor"). On information and belief, the recombinant methods used by GSK to produce Arzerra™ are similar to the recombinant methods used by MedImmune and Centocor to produce their monoclonal antibody products, Synagis®, ReoPro®, and Remicade®.

34. On information and belief, Genentech contends that the process and certain starting materials used to produce Arzerra™ infringe one or more claims of the Cabilly II patent.

35. For example, both GSK's Arzerra™ and MedImmune's Synagis® are produced by genetically engineering mammalian host cells to produce the desired antibody in cell culture. Arzerra™ is produced in a recombinant murine (mouse) cell line called NS0 using standard mammalian cell cultivation and purification techniques. On information and belief, Synagis® is also produced in a recombinant murine (mouse) cell line called NS0 using standard mammalian cell cultivation and purification techniques. Arzerra™ is an IgG1κ monoclonal antibody comprised of two heavy chains and two light chains. On information and belief, Synagis® is also an IgG1κ monoclonal antibody comprised of two heavy chains and two light chains. Like Arzerra™, on

information and belief, MedImmune's Synagis™ product is manufactured by Lonza. Since Genentech has previously enforced the Cabilly II patent against another Lonza customer that, on information and belief, uses the same NS0 cell line as Lonza uses for GSK, the same or similar transformation process as Lonza uses for GSK, and the same or similar manufacturing process as Lonza uses for GSK, GSK is informed and believes that Genentech contends that the methods used to produce Arzerra™ infringe one or more claims of the Cabilly II patent.

36. On information and belief, Genentech has also alleged that the corresponding recombinant methods and starting materials used to produce its Rituxan® antibody product fall within the scope of the Cabilly II patent. Like Arzerra™, Genentech's Rituxan® is produced by genetically engineering mammalian host cells to produce the desired antibody in cell culture. Like Arzerra™, Genentech's Rituxan® is an IgG1κ monoclonal antibody comprised of two heavy chains and two light chains. Like Arzerra™, Genentech's Rituxan® is directed against the CD20 antigen. Like Arzerra™, Lonza has manufactured Rituxan® for Genentech. If Genentech contends that the manufacturing process used by Lonza to produce Rituxan® for Genentech fell within the scope of the Cabilly II patent, then GSK is informed and believes that Genentech also contends that the manufacturing process used by Lonza to produce Arzerra™ for GSK also falls within the scope of the Cabilly II patent.

37. Since Defendants have consistently alleged that the use of well-known, conventional recombinant methods to produce monoclonal antibodies in mammalian cell culture is within the scope of claims of the Cabilly II patent and have asserted the patent against others who are similarly situated to GSK, Defendants' prior statements and conduct necessarily establish an actual and substantial dispute between GSK and Defendants regarding the invalidity, unenforceability, and noninfringement of claims of the Cabilly II patent. Therefore, GSK has a reasonable apprehension of suit by Genentech regarding the Cabilly II patent.

38. In addition to the statements and conduct directed at others, Defendants, including particularly Genentech, have made statements and engaged in conduct directed at GSK that create a real and immediate dispute between the parties regarding the Cabilly II patent.

39. Genentech has made public statements about pursuing an aggressive litigation policy to protect its products against competition and to protect against alleged infringement of the Cabilly II patent claims in its 2009 Form 10-K filing with the Securities and Exchange Commission. Genentech states:

> "Intellectual property protection of our products is crucial to our business. Loss of effective intellectual property protection could result in lost sales to competing products and loss of royalty payments (for example, royalty income associated with the **Cabilly patent**) from licenses. We are often involved in disputes over contracts and intellectual property, and we work to resolve these disputes in confidential negotiations or litigation. We expect legal challenges in this area to continue. We plan to continue to build upon and defend our intellectual property position." (emphasis added)

Genentech also states: "We have in the past been, are currently, **and may in the future be involved in material litigation** and other legal proceedings related to our proprietary rights, **such as the Cabilly patent litigation and reexamination** . . . ." (emphasis added)

40. In early 2009, Genentech made public statements specifically identifying Arzerra™ as an imminent competitor to Genentech's product Rituxan® in its filings with the Securities and Exchange Commission. In Genentech's 2009 Form 10-K, Genentech states:

> "**Rituxan may face future competition** in both hematology-oncology and RA **from Arzerra™ (ofatumumab)**, an anti-CD20 antibody being co-developed by Genmab A/S and GSK. Genmab and GSK recently presented positive results from their pivotal trial for CLL at the American Society of Hematology meeting. They announced on January 30, 2009 that they filed for approval for Arzerra™ for monotherapy use in refractory CLL." (emphasis added)

41. Taken together, Genentech's statements that it will enforce its intellectual property, specifically the Cabilly II patent, to defend its products against competing products, and its contention that GSK's Arzerra™ will be a competitor with Genentech's Rituxan® in hematology-oncology, establish that a real and immediate dispute exists between parties with adverse legal

interests concerning the Cabilly II patent. GSK therefore has a reasonable apprehension of suit by Genentech regarding the Cabilly II patent.

42. Genentech and GSK also have had repeated discussions and interactions relating to the Cabilly II patent that further establish the existence of a substantial dispute.

a. In 1991, one of GSK's predecessors-in-interest, Wellcome Foundation Ltd., entered into a license agreement with Genentech that included certain license rights relating to what later issued as the Cabilly II patent with respect to antibodies targeting CD4.

b. In 2002, Genentech and GSK entered into an agreement that provided for the parties to attempt to negotiate licenses under the Cabilly II patent for several different recombinant antibodies then in development by GSK. Licensing terms were ultimately never resolved and those antibody products have not yet been commercially sold in the United States.

c. In 2005, there were renewed discussions between Genentech and GSK regarding licensing the Cabilly II patent for a monoclonal antibody called mepolizumab that targeted the antigen IL-5. The parties were unable to reach agreement because Genentech proposed onerous terms that GSK believed were commercially unreasonable for the product, which was ultimately withdrawn from the regulatory approval process.

d. In 2005, a representative of Genentech, Tim Schwartz, asked GSK's counsel, Frank Grassler, to begin a discussion regarding a "Cabilly license for BEXXAR (anti-CD20) now that GSK has acquired the rights to this product." Like Arzerra™, BEXXAR is an antibody that targets the antigen CD20.

e. Mark Lemley, outside counsel for Genentech, and Sherry Knowles, Chief Patent Counsel for GSK are professional colleagues of mutual respect. In September 2008, following GSK's acquisition of rights to Arzerra™, Mr. Lemley told Ms. Knowles that he believed the Cabilly II patent would issue following reexamination and asked what GSK would then do about the Cabilly II patent. On information and belief, Mr.

Lemley believed the Cabilly II patent would be of interest to GSK, and his remarks conveyed that impression to Ms. Knowles.

43. Given Genentech's past positions and statements regarding the scope of the Cabilly II patent and the fact that GSK has begun marketing and selling Arzerra™ in the United States without a direct license from Genentech, the dispute between the parties is real and immediate.

44. The threat of litigation by Genentech to assert the Cabilly II patent against Arzerra™ is underscored by the parties' past legal disputes. Throughout the 1990s and early 2000s, Genentech and GSK's predecessors-in-interest were embroiled in multiple patent infringement actions, including at least one relating to recombinant antibody production.

Prior Action in the Southern District of Florida

45. To protect itself from the disruption of its commercial launch of Arzerra™ and secure a timely adjudication of its dispute with Genentech regarding the Cabilly II patent, on October 8, 2009, GSK filed a claim for declaratory judgment in the Southern District of Florida against Defendants. *See Glaxo Group Ltd. v. Genentech, Inc.*, Case No. 09-61608-CIV-LENARD/TURNOFF (S.D. Fla.). Defendants moved to dismiss the Florida complaint for lack of subject matter jurisdiction or, in the alternative, to transfer the action from the Southern District of Florida to the Central District of California. In their motion to dismiss, Defendants characterized GSK's action as "premature" because, among other things,

a. "At the time that plaintiffs (collectively 'GSK') filed this complaint, GSK had not received FDA approval for or made any commercial sales of Arzerra™ . . . ."

b. "GSK had no communications at all with Genentech or City of Hope regarding the Cabilly II patent in connection with Arzerra™."

Notably, Defendants did not state that they would not assert the Cabilly II patent against GSK's Arzerra™ product.

46. In their motion to transfer venue, Defendants challenged venue in Florida because, among other things, none of the parties were based in Florida. According to Defendants, "the locus of operative facts was in California" and "California is more convenient and less expensive for the parties and witnesses."

47. After filing the Florida complaint (and before filing this suit), GSK offered on two occasions to discuss with Genentech how the parties could resolve or otherwise dispose of the dispute relating to the Cabilly II patent. Genentech, however, failed to respond to GSK's offers to discuss the issue, nor did Genentech ever indicate that a license was unnecessary or that it did not intend to enforce the Cabilly II patent against GSK.

48. Rather than oppose Defendants' motion, GSK filed with the Southern District of Florida a notice of dismissal of its complaint under Federal Rule of Civil Procedure 41(a)(1)(A)(i), dismissing its complaint without prejudice.

49. GSK files the present action, in part, to address Defendants' objections to GSK's prior complaint for declaratory relief. The FDA has now approved Arzerra™ for commercial sale, and GSK has begun selling Arzerra™ in the United States. GSK has attempted to discuss the Cabilly II patent with Genentech in the context of Arzerra™, but Genentech declined to respond. To the extent Defendants objected to venue in Florida based on the convenience of the parties and witnesses, GSK files this suit in the Northern District of California, where Genentech has its headquarters and City of Hope has an established place of business.

50. Based on all of the circumstances, there is now an actual and justiciable controversy between GSK and Defendants with respect to whether the manufacture, importation, offer to sell, sale, or use of ofatumumab (Arzerra™) in the United States infringes any valid and enforceable claim of the Cabilly II patent.

FIRST CAUSE OF ACTION

PATENT INVALIDITY

51. GSK incorporates the allegations of paragraphs 1 through 50 as if fully set forth herein.

52. An actual and substantial controversy has arisen and now exists between the parties concerning the validity of the Cabilly II patent.

53. The Cabilly II patent is invalid because it is anticipated and/or obvious under 35 U.S.C. §§ 102 and 103 (2006).

54. The Cabilly II patent is invalid based on the judicially created doctrine of obviousness-type double patenting and/or under 35 U.S.C. §§ 101 and/or 103 (2006).

55. The Cabilly II patent is invalid under 35 U.S.C. § 112 (2006).

56. Claims 21-32 of the Cabilly II patent are invalid as being broadened in scope during reexamination in violation of 35 U.S.C. § 305 (2006).

57. GSK seeks a declaratory judgment that the Cabilly II patent is invalid under 35 U.S.C. §§ 101, 102, 103, 112, and 305 (2006) and/or under the judicially created doctrine of obviousness-type double patenting.

SECOND CAUSE OF ACTION

NON-INFRINGEMENT

58. GSK incorporates the allegations of paragraphs 1 through 57 as if fully set forth herein.

59. An actual controversy has arisen and now exists between the parties concerning whether GSK's manufacturing process or importation or sale of ofatumumab (Arzerra™) infringes any valid and enforceable claim of the Cabilly II patent.

60. GSK seeks a declaratory judgment that making, using, importing, offering to sell, and selling ofatumumab (Arzerra™) does not and will not infringe any valid and enforceable claim of the Cabilly II patent.

THIRD CAUSE OF ACTION

PROSECUTION LACHES

61. GSK incorporates the allegations of paragraphs 1 through 60 as if fully set forth herein.

62. An actual controversy has arisen and now exists between the parties concerning the enforceability of the Cabilly II patent.

63. The Cabilly II patent is unenforceable under the doctrine of prosecution laches. The Cabilly II patent issued after an unreasonable and unexplained delay in the interference proceedings between the Cabilly II application and the Boss patent. Genentech also unreasonably delayed the

prosecution of claims 21, 22, 27-30, and 32, which were filed as part of the Cabilly II application in 1983 but did not issue until 2001.

64. GSK seeks a declaratory judgment that the Cabilly II patent is unenforceable due to prosecution laches.

PRAYER FOR RELIEF

WHEREFORE, GSK requests that judgment be entered in favor of GSK and against Defendants Genentech and City of Hope:

1. Declaring the Cabilly II patent invalid;

2. Declaring the Cabilly II patent unenforceable;

3. Declaring that the manufacture, use, sale, offer to sell, or importation of GSK's ofatumumab (Arzerra™) product does not infringe any valid and enforceable claim of the Cabilly II patent;

4. Enjoining Genentech and City of Hope from enforcing the Cabilly II patent;

5. Awarding GSK its costs and attorneys' fees; and

6. Awarding GSK such other relief as the Court deems just and proper.

Date: February 17, 2010

Respectfully submitted,

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EXHIBIT A

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UNITED STATES DISTRICT COURT

FOR THE NORTHERN DISTRICT OF CALIFORNIA

SAN FRANCISCO DIVISION

JSW

CV 10 675

GLAXO GROUP LIMITED and GLAXOSMITHKLINE LLC,

Plaintiffs,

vs.

GENENTECH, INC., and CITY OF HOPE,

Defendants.

Case No.:

COMPLAINT FOR DECLARATORY JUDGMENT OF INVALIDITY, UNENFORCEABILITY, AND NONINFRINGEMENT

Plaintiffs Glaxo Group Limited and GlaxoSmithKline LLC (collectively, "GSK"), for their Complaint against Genentech, Inc. and City of Hope (collectively, "Defendants"), allege as follows:

NATURE OF THE CASE

1. GSK seeks a declaration that U.S. Patent 6,331,415 titled "Methods of Producing Immunoglobulins, Vectors and Transformed Host Cells for Use Therein" (the "Cabilly II patent" attached as Exhibit A), including the *Ex Parte* Reexamination Certificate issued pursuant to Reexamination Nos. 90/007,542 and 90/007,859 (attached as Exhibit B), is invalid, unenforceable, and not infringed by the manufacture, use, sale, offer to sell, or importation of GSK's ofatumumab (Arzerra™) antibody product.

2. GSK recently began marketing and selling Arzerra™ in the United States for the treatment of patients whose chronic lymphocytic leukemia ("CLL") is refractory to previous

US006331415B1

(12) **United States Patent**
Cabilly et al.

(10) **Patent No.: US 6,331,415 B1**
(45) **Date of Patent: Dec. 18, 2001**

(54) **METHODS OF PRODUCING IMMUNOGLOBULINS, VECTORS AND TRANSFORMED HOST CELLS FOR USE THEREIN**

(75) Inventors: **Shmuel Cabilly**, Monrovia; **Herbert L. Heyneker**, Burlingame; **William E. Holmes**, Pacifica; **Arthur D. Riggs**, La Verne; **Ronald B. Wetzel**, San Francisco, all of CA (US)

(73) Assignee: **Genentech, Inc.**, South San Francisco, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **07/205,419**

(22) Filed: **Jun. 10, 1988**

Related U.S. Application Data

(63) Continuation of application No. 06/483,457, filed on Apr. 8, 1983, now Pat. No. 4,816,567.

(51) **Int. Cl.**[7] **C12N 15/13**; C12N 15/00; C12N 15/63

(52) **U.S. Cl.** **435/69.6**; 435/69.1; 435/69.7; 435/70.1; 435/70.21; 435/71.1; 435/71.2; 435/320; 435/252.1; 435/252.3; 435/252.33; 435/254.11; 435/254.2; 435/254.21; 435/455; 435/471; 435/483; 435/485

(58) **Field of Search** 435/69.1, 69.7, 435/71.1, 70.1, 71.2, 320, 261, 252.1, 252.3, 81, 55, 56, 69.6, 252.33, 254.21, 483

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(List continued on next page.)

Primary Examiner—Phillip Gambel
(74) *Attorney, Agent, or Firm*—Burns, Doane, Swecker & Mathis, LLP

(57) **ABSTRACT**

The invention relates to processes for producing an immunoglobulin or an immunologically functional immunoglobulin fragment containing at least the variable domains of the immunoglobulin heavy and light chains. The processes can use one or more vectors which produce both the heavy and light chains or fragments thereof in a single cell. The invention also relates to the vectors used to produce the immunoglobulin or fragment, and to cells transformed with the vectors.

36 Claims, 19 Drawing Sheets

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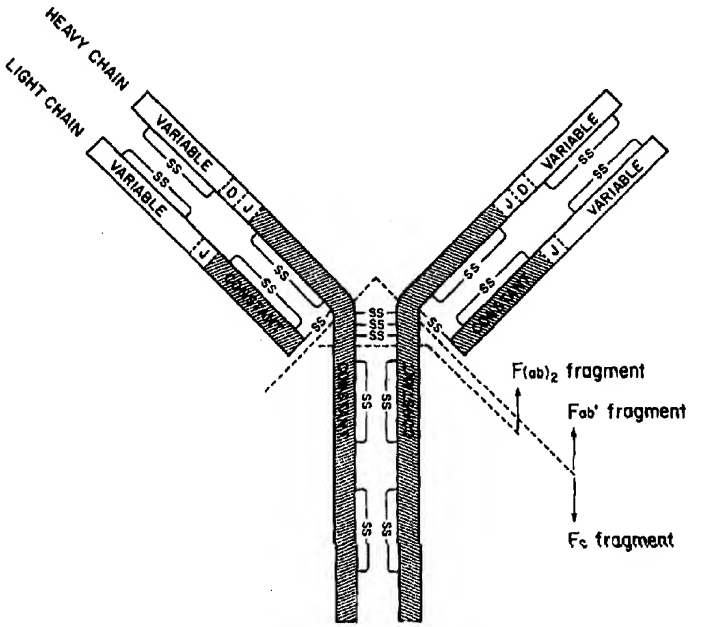

HEAVY CHAIN
LIGHT CHAIN
VARIABLE
D
J
SS
CONSTANT
F(ab)2 fragment
Fab' fragment
Fc fragment

Fig. 1.

```
                                                                                                                        haeIII
                                                  tthIII                                 tthIII         hphI       haeI
1     GTTGCTGTGG TTGTCTGGTG TTGAAGGAGA CATTGTGATG ACCCAGTCTC ACAAATTCAT GTCCACATCA GTAGGAGACA GGGTCAGCAT CACCTGCAAG
      CAACGACACC AACAGACCAC AACTTCCTCT GTAACACTAC TGGGTCAGAG TGTTTAAGTA CAGGTGTAGT CATCCTCTGT CCCAGTCGTA GTGGACGTTC
                                                                                                    sfaNI

                                                                                                  scrFI
                   fnu4HI        scrFI                 scrFI                                      ncII
            fokI   bbv           ecoRII                ecoRII                                     hpaII       hinfI
101   GCCAGTCAGG ATGTGGGTGC TGCTATAGCC TGGTATCAAC AGAAACCAGG ACAATCTCCT AAACTACTGA TTTACTGGGC ATCCACCCGG CACACTGGAG
      CGGTCAGTCC TACACCCACG ACGATATCGG ACCATAGTTG TCTTTGGTCC TGTTAGAGGA TTTGATGACT AAATGACCCG TAGGTGGGCC GTGTGACCTC
                                                                                     fokI     sfaNI

                        xhoII
         sau3A           sau3A
         dpnI            dpnI                              hphI                                                 hincII
201   TCCCTGATCG CTTCACAGGC AGTGGATCTG GGACAGATTT CACTCTCACC ATTAGCAATG TGCAGTCTGA TGACTTGGCA GATTATTTCT GTCAACAATA
      AGGGACTAGC GAAGTGTCCG TCACCTAGAC CCTGTCTAAA GTGAGAGTGG TAATCGTTAC ACGTCAGACT ACTGAACCGT CTAATAAAGA CAGTTGTTAT

                                      sau96                           fnu4HI                                     hpaI
                  mnlI                avaII   aluI    aluI sfaNI      bbv                 mboII                 hincII
301   TAGCGGGTAT CCTCTCACGT TCGGTGCTGG GACCAAGCTG GAGCTGAAAC GGGCTGATGC TGCACCAACT GTATCCATCT TCCCACCATC CAGTGAGCAG
      ATCGCCCATA GGAGAGTGCA AGCCACGACC CTGGTTCGAC CTCGACTTTG CCCGACTACG ACGTGGTTGA CATAGGTAGA AGGGTGGTAG GTCACTCGTC
                                                                                          fokI

                       mnlI
                  mnlI  ddeI                        xmnI                                   mboII                  acyI
401   TTAACATCTG GAGGTGCCTC AGTCGTGTGC TTCTTGAACA ACTTCTACCC CAAAGACATC AATGTCAAGT GGAAGATTGA TGGCAGTGAA CGACAAAATG
      AATTGTAGAC CTCCACGGAG TCAGCACACG AAGAACTTGT TGAAGATGGG GTTTCTGTAG TTACAGTTCA CCTTCTAACT ACCGTCACTT GCTGTTTTAC
```

Fig. 2A.

therapies (fludarabine and alemtuzumab). GSK brings this action to lift the cloud created by the imminent threat of Defendants' enforcement of the Cabilly II patent against GSK. Without declaratory relief, the threat of enforcement of the Cabilly II patent poses a substantial risk of injury to GSK as well as the patients, nurses, and doctors now using Arzerra™ for treatment. The continued existence and enforcement of this invalid and unenforceable patent impedes not only the development and sale of Arzerra™, but also the development and sale of other life-saving recombinant antibody products.

3. Defendants have asserted that the Cabilly II patent broadly covers the use of certain well-known, conventional recombinant methods to produce any antibody product in any type of host cell. Defendants have filed infringement claims under the Cabilly II patent against companies who have made and sold antibody products that were produced using recombinant methods similar to the recombinant methods used by GSK to make Arzerra™. Defendant Genentech, Inc. has specifically identified GSK's Arzerra™ antibody product as a potential competitor to one of Genentech's own products, and has stated that it expects to be involved in future litigation relating to the enforcement of the Cabilly II patent. During GSK's dealings with Genentech, Genentech has repeatedly taken the position that GSK requires a license under the Cabilly II patent to make and sell a variety of different antibody products, including products produced by the same or similar process as Arzerra™. As recently as the Fall of 2008, after GSK acquired rights to Arzerra™, counsel for Genentech inquired what GSK would do about the Cabilly II patent. Given Defendants' past acts and statements and GSK's sale of Arzerra™ in the United States, a real, immediate, and substantial dispute exists between the parties concerning the Cabilly II patent for which GSK now seeks declaratory relief.

THE PARTIES

4. Plaintiff Glaxo Group Limited d/b/a GlaxoSmithKline is an English corporation having a principal place of business at Glaxo Wellcome House, Berkley Avenue, Greenford, Middlesex, UB6 0NN, United Kingdom.

5. Plaintiff GlaxoSmithKline LLC is a Delaware limited liability company having a principal place of business at One Franklin Plaza, Philadelphia, Pennsylvania, 19102.

sau3A
dpnI
bclI
fnu4HI
bbv
mnlI hincII
aluI
hgaI
GCGTCCTGAA CAGTTGGACT GATCAGGACA GCAAAGACAG CACCTACAGC ATGAGCAGCA CCCTCACGTT GACCAAGGAC GAGTATGAAC GACATAACAG
CGCAGGACTT GTCAACCTGA CTAGTCCTGT CGTTTCTGTC GTGGATGTCG TACTCGTCGT GGGAGTGCAA CTGGTTCCTG CTCATACTTG CTGTATTGTC
mnlI
haeIII
haeI
hphI
aluI
sau96
ddeI
hgaI
avaII
acyI
CTATACCTGT GAGGCCACTC ACAAGACATC AACTTCACCC ATTGTCAAGA GCTTCAACAG GAATGAGTGT TAGAGACAAA GGTCCTGAGA CGCCACCACC
GATATGGACA CTCCGGTGAG TGTTCTGTAG TTGAAGTGGG TAACAGTTCT CGAAGTTGTC CTTACTCACA ATCTCTGTTT CCAGGACTCT GCGGTGGTGG
aluI
aluI
mboII
ddeI
mnlI
mnlI
hgiA
mnlI
mnlI
AGCTCCCCAG CTCCATCCTA TCTTCCCTTC TAAGGTCTTG GAGGCTTCCC CACAAGCGAC CTACCACTGT TGCGGTGCTC CAAACCTCCT CCCCACCTCC
TCGAGGGGTC GAGGTAGGAT AGAAGGGAAG ATTCCAGAAC CTCCGAAGGG GTGTTCGCTG GATGGTGACA ACGCCACGAG GTTTGGAGGA GGGGTGGAGG
fokI
mnlI
mnlI
mnlI
xmnI
hinfI
TTCTCCTCCT CCTCCCTTTC CTTGGCTTTT ATCATGCTAA TATTTGCAGA AAATATTCAA TAAAGTGAGT CTTTGCACTT GA
AAGAGGAGGA GGAGGGAAAG GAACCGAAAA TAGTACGATT ATAAACGTCT TTTATAAGTT ATTTCACTCA GAAACGTGAA CT
nucleotides: 882

Fig. 2B.

```
   -9                                      1                                       10                                      20
   leu leu trp leu ser gly val glu gly asp ile val met thr gln ser his lys phe met ser thr ser val gly asp arg val ser
G  UUG CUG UGG UUG UCU GGU GUU GAA GGA GAC AUU GUG AUG ACC CAG UCU CAC AAA UUC AUG UCC ACA UCA GUA GGA GAC AGG GUC AGC

                                     30                                      40                                      50
ile thr cys lys ala ser gln asp val gly ala ala ile ala trp tyr gln gln lys pro gly gln ser pro lys leu leu ile tyr trp
AUC ACC UGC AAG GCC AGU CAG GAU GUG GGU GCU GCU AUA GCC UGG UAU CAA CAG AAA CCA GGA CAA UCU CCU AAA CUA CUG AUU UAC UGG

                                     60                                      70                                      80
ala ser thr arg his thr gly val pro asp arg phe thr gly ser gly ser gly thr asp phe thr leu thr ile ser asn val gln ser
GCA UCC ACC CGG CAC ACU GGA GUC CCU GAU CGC UUC ACA GGC AGU GGA UCU GGG ACA GAU UUC ACU CUC ACC AUU AGC AAU GUG CAG UCU

                                     90                                      100                                     110
asp asp leu ala asp tyr phe cys gln gln tyr ser gly tyr pro leu thr phe gly ala gly thr lys leu glu leu lys arg ala asp
GAU GAC UUG GCA GAU UAU UUC UGU CAA CAA UAU AGC GGG UAU CCU CUC ACG UUC GGU GCU GGG ACC AAG CUG GAG CUG AAA CGG GCU GAU

                                     120                                     130                                     140
ala ala pro thr val ser ile phe pro pro ser ser glu gln leu thr ser gly gly ala ser val val cys phe leu asn asn phe tyr
GCU GCA CCA ACU GUA UCC AUC UUC CCA CCA UCC AGU GAG CAG UUA ACA UCU GGA GGU GCC UCA GUC GUG UGC UUC UUG AAC AAC UUC UAC

                                     150                                     160                                     170
pro lys asp ile asn val lys trp lys ile asp gly ser glu arg gln asn gly val leu asn ser trp thr asp gln asp ser lys asp
CCC AAA GAC AUC AAU GUC AAG UGG AAG AUU GAU GGC AGU GAA CGA CAA AAU GGC GUC CUG AAC AGU UGG ACU GAU CAG GAC AGC AAA GAC

                                     180                                     190                                     200
ser thr tyr ser met ser ser thr leu thr leu thr lys asp glu tyr glu arg his asn ser tyr thr cys glu ala thr his lys thr
AGC ACC UAC AGC AUG AGC AGC ACC CUC ACG UUG ACC AAG GAC GAG UAU GAA CGA CAU AAC AGC UAU ACC UGU GAG GCC ACU CAC AAG ACA

                                     210                     214
ser thr ser pro ile val lys ser phe asn arg asn glu cys AM
UCA ACU UCA CCC AUU GUC AAG AGC UUC AAC AGG AAU GAG UGU UAG AGACAAAGGUCCUGAGACGCCACCACCAGCUCCCCAGCUCCAUCCUAUCUUCCCUUCUAA

GGUCUUGGAGGCUUCCCCACAAGCGACCUACCACUGUUGCGGUGCUCCAAACCUCCUCCCCACCUCCUUCUCCUCCUCCUCCCUUUCCUUGGCUUUUAUCAUGCUAAUAUUUGCAGAAAA

UAUUCAAUAAAGUGAGUCUUUGCACUUGA
```

Fig. 3

```
                        sau96                    ddeI
        hinfI           avaII mnlI                  aluI                  ahaIII          sfaNI
1       GAGTCAGCAC TGAACACGGA CCCCTCACGA TGAACTTCGG GCTCAGCTTG ATTTACCTTG TCCTTGTTTT AAAAGTTGTC CAGTGTGAAG TGATGCTGGT
        CTCAGTCGTG ACTTGTGCCT GGGGAGTGCT ACTTGAAGCC CGAGTCGAAC TAAATGGAAC AGGAACAAAA TTTTCAACAG GTCACACTTC ACTACGACCA

                                    scrFI   sau96
                                        mnlI                   fnu4HI
         hinfI      hinfI           ecoRII  avaII              bbv mnlI  hinfI
101     GGAGTCTGGG GGAGTCTTAA TGGAGCCTGG AGGGTCCCTG AAACTCTCCT GTGCAGCCTC TGGATTCACT TTCAGTAGAT ATGCCATGTC TTGGGTTCGC
        CCTCAGACCC CCTCAGAATT ACCTCGGACC TCCCAGGGAC TTTGAGAGGA CACGTCGGAG ACCTAAGTGA AAGTCATCTA TACGGTACAG AACCCAAGCG

             hpaII   mnlI                                                                            hphI
         hinfI      mboII                                                                           hinfI
201     CAGACTCCGG AGAAGAGGCT GGAGTGGGTC GCAACCATTA GTAGTGGTGG TAGTTCACAC CTTCCATCCA GACAGTGTGA AGGGCGATTC ACCATCTCCA
        GTCTGAGGCC TCTTCTCCGA CCTCACCCAG CGTTGGTAAT CATCACCACC ATCAAGTGTG GAAGGTAGGT CTGTCACACT TCCCGCTAAG TGGTAGAGGT
                                                                 fokI

                                                   mnlI    mnlI
                              rsaI                ddeI   ddeI       haeIII                  mnlI
301     GAGACAATGC CAAGAACACC CTGTACCTGC AAATGAGCAG TCTGAGGTCT GAGGACACGG CCATGTATTA CTGTGCAAGA CCCCCTCTTA TTTCGTTAGT
        CTCTGTTACG GTTCTTGTGG GACATGGACG TTTACTCGTC AGACTCCAGA CTCCTGTGCC GGTACATAAT GACACGTTCT GGGGGAGAAT AAAGCAATCA

                                                                                                         xhoII
                                                                                                       scrFI
                                                                                                   sau96   sau3A
                                          mnlI            mnlI                                         ecoRII
                                           ddeI  hphI      ddeI                                    haeIII  dpnI
401     AGCGGACTAT GCTATGGACT ACTGGGGTCA AGGAACCTCA GTCACCGTCT CCTCAGCCAA AACGACACCC CCATCTGTCT ATCCACTGGC CCCTGGATCT
        TCGCCTGATA CGATACCTGA TGACCCCAGT TCCTTGGAGT CAGTGGCAGA GGAGTCGGTT TTGCTGTGGG GGTAGACAGA TAGGTGACCG GGGACCTAGA
```

Fig. 4A.

```
                                     scrFI                                                      xhoII
                           ncol   sfaNI    fokI                                                  sau3A
           fnu4HI              hphI  ecoRII    scrFI                                 scrFI       dpnI
           bbv                 bstEII          ecoRII              ddeI              ecoRII     bamHI
501        GCTGCCCAAA CTAACTCCAT GGTGACCCTG GGATGCCTGG TCAAGGGCTA TTTCCCTGAG CCAGTGACAG TGACCTGGAA CTCTGGATCC CTGTCCAGCG
           CGACGGGTTT GATTGAGGTA CCACTGGGAC CCTACGGACC AGTTCCCGAT AAAGGGACTC GGTCACTGTC ACTGGACCTT GAGACCTAGG GACAGGTCGC

                                                          fnu4HI                      sau96
                          pvuII                           bbv  ddeI               mnlI
             hgiA           aluI     pstI       mnlI   ddeI   aluI            mnlI       haeIII           hphI
601        GTGTGCACAC CTTCCCAGCT GTCCTGCAGT CTGACCTCTA CACTCTGAGC AGCTCAGTGA CTGTCCCCTC CAGCCCTCGG CCCAGCGAGA CCGTCACCTG
           CACACGTGTG GAAGGGTCGA CAGGACGTCA GACTGGAGAT GTGAGACTCG TCGAGTCACT GACAGGGGAG GTCGGGAGCC GGGTCGCTCT GGCAGTGGAC

                       scrFI
                          haeIII
                       ncII     fnu4HI                        scrFI
                  bglI   hpaII    bbv                         ecoRII                        ndeI  rsaI
701        CAACGTTGCC CACCCGGCCA GCAGCACCAA GGTGGACAAG AAAATTGTGC CCAGGGATTG TGGTTGTAAG CCTTGCATAT GTACAGTCCC AGAAGTATCA
           GTTGCAACGG GTGGGCCGGT CGTCGTGGTT CCACCTGTTC TTTTAACACG GGTCCCTAAC ACCAACATTC GGAACGTATA CATGTCAGGG TCTTCATAGT

                                                                  mstII                                        sau3A
                                                  hphI           hinfI                                         dpnI   mnlI
              mboII  mboII                    fokI hgiA               ddeI                 accI          fokI    avaI
801        TCTGTCTTCA TCTTCCCCCC AAAGCCCAAG GATGTGCTCA CCATTACTCT GACTCCTAAG GTCACGTGTG TTGTGGTAGA CATCAGCAAG GATGATCCCG
           AGACAGAAGT AGAAGGGGGG TTTCGGGTTC CTACACGAGT GGTAATGAGA CTGAGGATTC CAGTGCACAC AACACCATCT GTAGTCGTTC CTACTAGGGC

                                                                     smaI
                                                                      scrFI
                                                                     scrFI
                                                                      ncII
                                                                     ncII
            sau96     pvuII                  mnlI       ddeI          hpaII
            avaII      aluI                     hgiA   aluI  hgaI    avaI  mnlI                    ddeI
901        AGGTCCAGTT CAGCTGGTTT GTAGATGATG TGGAGGTGCA CACAGCTCAG ACGCAACCCC GGGAGGAGCA GTTCAACAGC ACTTTCCGCT CAGTCAGTGA
           TCCAGGTCAA GTCGACCAAA CATCTACTAC ACCTCCACGT GTGTCGAGTC TGCGTTGGGG CCCTCCTCGT CAAGTTGTCG TGAAAGGCGA GTCAGTCACT
```

Fig. 4B.

```
                                                                                 fnu4HI
                  scrFI                                                          bbv
                  ecoRII                                          hincII           aluI                    taqI
1001  ACTTCCCATC ATGCACCAGG ACTGGCTCAA TGGCAAGGAG TTCAAATGCA GGGTCAACAG TGCAGCTTTC CCTGCCCCCA TCGAGAAAAC CATCTCCAAA
      TGAAGGGTAG TACGTGGTCC TGACCGAGTT ACCGTTCCTC AAGTTTACGT CCCAGTTGTC ACGTCGAAAG GGACGGGGGT AGCTCTTTTG GTAGAGGTTT

                                                                              haeIII
                                                                              haeI
                                       rsaI         mnlI                      balI
1101  ACCAAAGGCA GACCGAAGGC TCCACAGGTG TACACCATTC CACCTCCCAA GGAGCAGATG GCCAAGGATA AAGTCAGTCT GACCTGCATG ATAACAGACT
      TGGTTTCCGT CTGGCTTCCG AGGTGTCCAC ATGTGGTAAG GTGGAGGGTT CCTCGTCTAC CGGTTCCTAT TTCAGTCAGA CTGGACGTAC TATTGTCTGA

                                                   fnu4HI
      mboII      mboII                             bbv                                ddeI
1201  TCTTCCCTGA AGACATTACT GTGGAGTGGC AGTGGAATGG GCAGCCAGCG GAGAACTACA AGAACACTCA GCCCATCATG AACACGAATG GCTCTTACTT
      AGAAGGGACT TCTGTAATGA CACCTCACCG TCACCTTACC CGTCGGTCGC CTCTTGATGT TCTTGTGAGT CGGGTAGTAC TTGTGCTTAC CGAGAATGAA

                                                                                            sau96
                                                                                          mnlI                 mboII
       accI        aluI          mboII        mnlI            hphI                           haeIII          ddeI
1301  CGTCTACAGC AAGCTCAATG TGCAGAAGAG CAACTGGGAG GCAGGAAATA CTTTCACCTG CTCTGTGTTA CATGAGGGCC TGCACAACCA CCATACTGAG
      GCAGATGTCG TTCGAGTTAC ACGTCTTCTC GTTGACCCTC CGTCCTTTAT GAAAGTGGAC GAGACACAAT GTACTCCCGG ACGTGTTGGT GGTATGACTC

                             scrFI      sau3A                        sau96
         mnlI                ecoRII     dpnI                  mnlI   avaII      hinfI        mnlI      mnlI
1401  AAGAGCCTCT CCCACTCTCC TGGTAAATGA TCCCAGTGTC CTTGGAGCCC TCTGGTCCTA CAGGACTCTG ACACCTACCT CCACCCCTCC CTGTATAAAT
      TTCTCGGAGA GGGTGAGAGG ACCATTTACT AGGGTCACAG GAACCTCGGG AGACCAGGAT GTCCTGAGAC TGTGGATGGA GGTGGGGAGG GACATATTTA

1501  AAAGCACCCA GCACTGCCTT GGGAAAAA
      TTTCGTGGGT CGTGACGGAA CCCTTTTT
```

Fig.4C.

GAGUCAGCACUGAAACACGGACCCCUCACG
met asn phe gly leu ser leu ile tyr
-10
leu val leu val leu lys val val gln cys glu
1
val met leu val glu ser gly gly val leu met glu pro gly gly ser leu lys leu ser cys ala ala ser gly phe thr phe ser arg
10
20
30
tyr ala met ser trp val arg gln thr pro glu lys arg leu glu trp val ala thr ile ser ser gly gly ser ser his leu pro ser
40
50
60
arg gln cys glu gly arg phe thr ile ser arg asp asn ala lys asn thr leu tyr leu gln met ser ser leu arg ser glu asp thr
70
80
90
ala met tyr tyr cys ala arg pro pro leu ile ser leu val ala asp tyr ala met asp tyr trp gly gln gly thr ser val thr val
100
110
120
ser ser ala lys thr thr pro pro ser val tyr pro leu ala pro gly ser ala ala gln thr asn ser met val thr leu gly cys leu
130
140
150
val lys gly tyr phe pro glu pro val thr val thr trp asn ser gly ser leu ser ser gly val his thr phe pro ala val leu gln
160
170
180
ser asp leu tyr thr leu ser ser ser val thr val pro ser ser pro arg pro ser glu thr val thr cys asn val ala his pro ala
190
200
210
ser ser thr lys val asp lys lys ile val pro arg asp cys gly cys lys pro cys ile cys thr val pro glu val ser ser val phe
220
230
240

Fig. 5A.

```
                                250                                     260                                     270
ile phe pro pro lys pro lys asp val leu thr ile thr leu thr pro lys val thr cys val val val asp ile ser lys asp asp pro
AUC UUC CCC CCA AAG CCC AAG GAU GUG CUC ACC AUU ACU CUG ACU CCU AAG GUC ACG UGU GUU GUG GUA GAC AUC AGC AAG GAU GAU CCC

                                280                                     290                                     300
glu val gln phe ser trp phe val asp asp val glu val his thr ala gln thr gln pro arg glu glu gln phe asn ser thr phe arg
GAG GUC CAG UUC AGC UGG UUU GUA GAU GAU GUG GAG GUG CAC ACA GCU CAG ACG CAA CCC CGG GAG GAG CAG UUC AAC AGC ACU UUC CGC

                                310                                     320                                     330
ser val ser glu leu pro ile met his gln asp trp leu asn gly lys glu phe lys cys arg val asn ser ala ala phe pro ala pro
UCA GUC AGU GAA CUU CCC AUC AUG CAC CAG GAC UGG CUC AAU GGC AAG GAG UUC AAA UGC AGG GUC AAC AGU GCA GCU UUC CCU GCC CCC

                                340                                     350                                     360
ile glu lys thr ile ser lys thr lys gly arg pro lys ala pro gln val tyr thr ile pro pro pro lys glu gln met ala lys asp
AUC GAG AAA ACC AUC UCC AAA ACC AAA GGC AGA CCG AAG GCU CCA CAG GUG UAC ACC AUU CCA CCU CCC AAG GAG CAG AUG GCC AAG GAU

                                370                                     380                                     390
lys val ser leu thr cys met ile thr asp phe phe pro glu asp ile thr val glu trp gln trp asn gly gln pro ala glu asn tyr
AAA GUC AGU CUG ACC UGC AUG AUA ACA GAC UUC UUC CCU GAA GAC AUU ACU GUG GAG UGG CAG UGG AAU GGG CAG CCA GCG GAG AAC UAC

                                400                                     410                                     420
lys asn thr gln pro ile met asn thr asn gly ser tyr phe val tyr ser lys leu asn val gln lys ser asn trp glu ala gly asn
AAG AAC ACU CAG CCC AUC AUG AAC ACG AAU GGC UCU UAC UUC GUC UAC AGC AAG CUC AAU GUG CAG AAG AGC AAC UGG GAG GCA GGA AAU

                                430                                     440                         447
thr phe thr cys ser val leu his glu gly leu his asn his his thr glu lys ser leu ser his ser pro gly lys OP
ACU UUC ACC UGC UCU GUG UUA CAU GAG GGC CUG CAC AAC CAC CAU ACU GAG AAG AGC CUC UCC CAC UCU CCU GGU AAA UGA UCCCAGUGUCCU

UGGAGCCCUCUGGUCCUACAGGACUCUGACACCUACCUCCACCCCUCCCUGUAUAAAUAAAGCACCCAGCACUGCCUUGGGAAAAA
```

Fig. 5B.

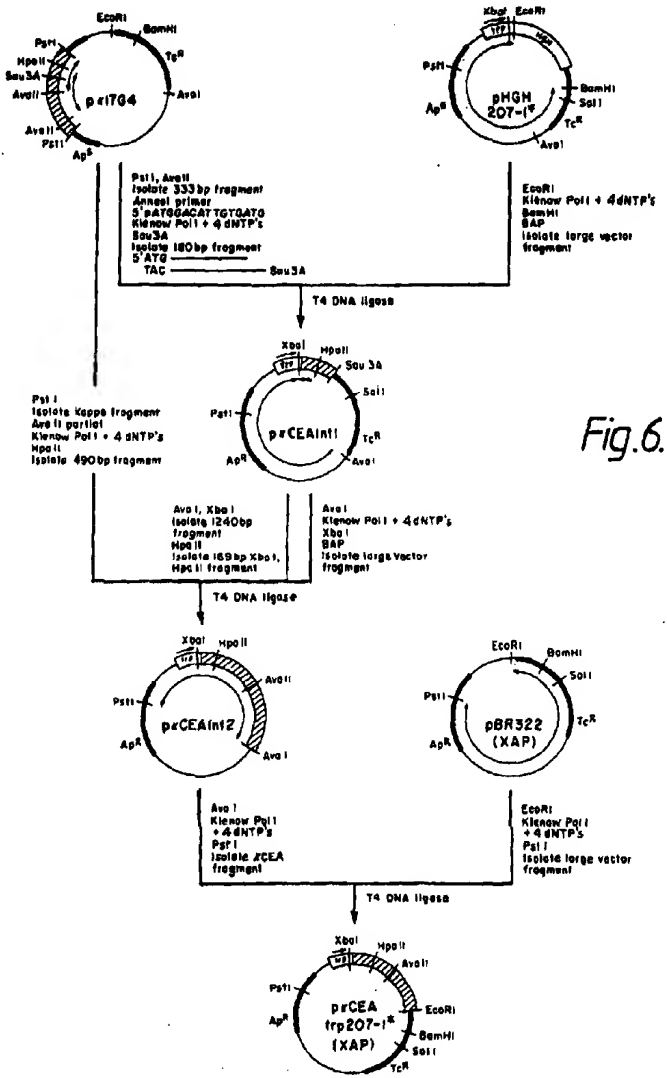

EcoRI
BamHI
PstI
HpaII
Sau3A
AvaII
TcR
AvaI
AvaII
PstI
ApR
prIT64
XbaI
EcoRI
trp
SD
PstI
BamHI
SalI
ApR
pHGH 207-1R
TcR
AvaI
PstI, AvaII
Isolate 333 bp fragment
Anneal primer
5'pATGGACATTGTGATG
Klenow Pol I + 4 dNTP's
Sau3A
Isolate 180 bp fragment
5'ATG
TAC
Sau3A
EcoRI
Klenow Pol I + 4dNTP's
BamHI
BAP
Isolate large vector fragment
T4 DNA ligase
XbaI
HpaII
trp
Sau 3A
SalI
PstI
TcR
ApR
AvaI
prCEAint1
PstI
Isolate Kappa fragment
AvaII partial
Klenow Pol I + 4 dNTP's
HpaII
Isolate 490 bp fragment
AvaI, XbaI
Isolate 1240 bp
fragment
HpaII
Isolate 169 bp XbaI,
HpaII fragment
AvaI
Klenow Pol I + 4dNTP's
XbaI
BAP
Isolate large vector
fragment
T4 DNA ligase
XbaI
HpaII
trp
AvaII
PstI
prCEAint2
ApR
AvaI
EcoRI
BamHI
SalI
PstI
pBR322 (XAP)
TcR
ApR
AvaI
Klenow Pol I
+ 4 dNTP's
PstI
Isolate /CEA
fragment
EcoRI
Klenow Pol I
+ 4 dNTP's
PstI
Isolate large vector
fragment
T4 DNA ligase
XbaI
HpaII
AvaII
PstI
prCEA
trp207-1*
(XAP)
EcoRI
ApR
BamHI
SalI
TcR

Fig.6.

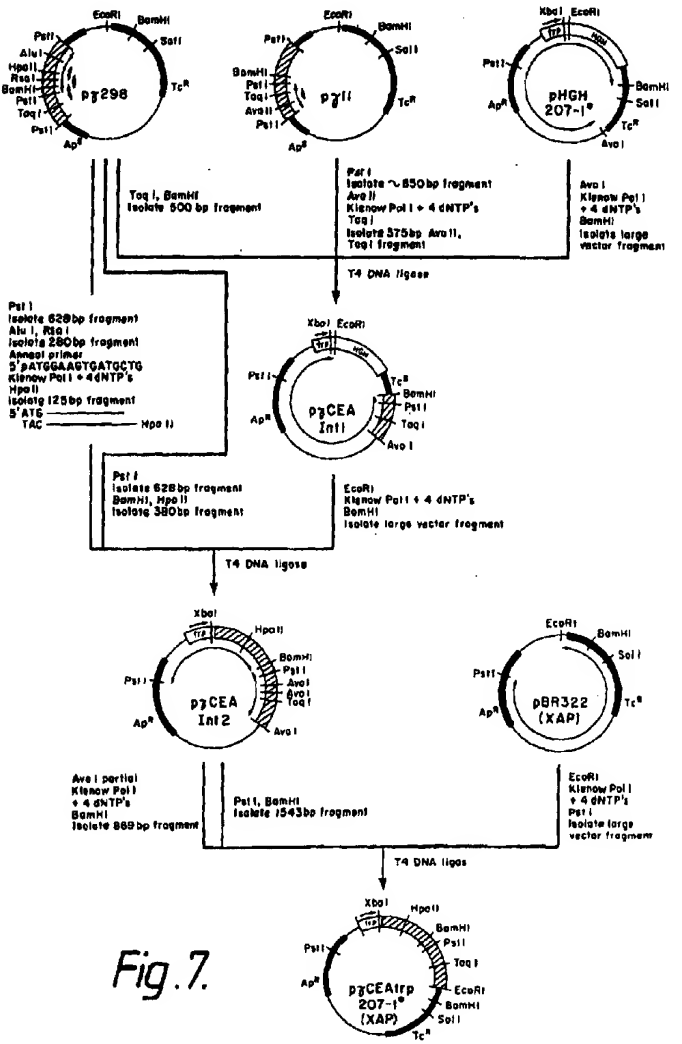

pγ298
pγII
pHGH 207-1®
Taq I, BamHI
Isolate 500 bp fragment
Pst I
Isolate ~850bp fragment
Ava II
Klenow Pol I + 4 dNTP's
Taq I
Isolate 375bp Ava II, Taq I fragment
Ava I
Klenow Pol I + 4 dNTP's
BamHI
Isolate large vector fragment
T4 DNA ligase
Pst I
Isolate 628bp fragment
Alu I, Rsa I
Isolate 280bp fragment
Anneal primer
5'PATGGAAGTGATGCTG
Klenow Pol I + 4dNTP's
Hpa II
Isolate 125bp fragment
5'ATG
TAC
Hpa II
pγCEA Int1
Pst I
Isolate 628bp fragment
BamHI, Hpa II
Isolate 380bp fragment
EcoRI
Klenow Pol I + 4 dNTP's
BamHI
Isolate large vector fragment
T4 DNA ligase
pγCEA Int2
pBR322 (XAP)
Ava I partial
Klenow Pol I
+ 4 dNTP's
BamHI
Isolate 869 bp fragment
Pst I, BamHI
Isolate 1543bp fragment
EcoRI
Klenow Pol I
+ 4 dNTP's
Pst I
Isolate large vector fragment
T4 DNA ligase
pγCEAIrp 207-1® (XAP)

Fig. 7.

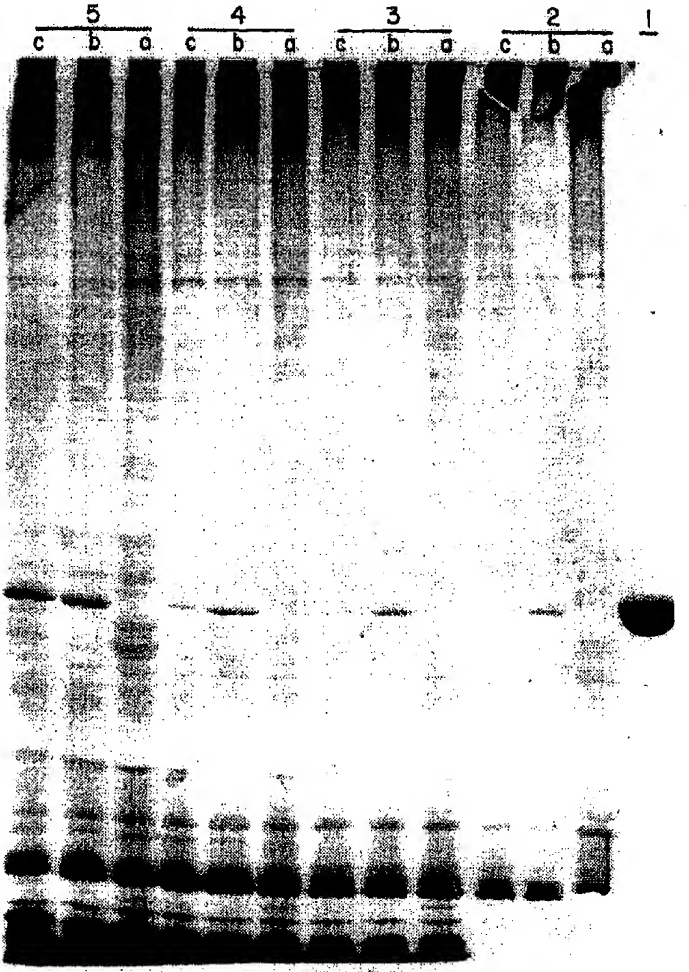


Fig. 8A.

6. Defendant Genentech, Inc. ("Genentech") is a Delaware corporation having its principal place of business in South San Francisco, California.

7. City of Hope is a California not-for-profit organization having its principal place of business in Duarte, California. On information and belief, City of Hope has a place of business in this District at 55 Hawthorne Street, Suite 450, San Francisco, California, 94105.

8. On information and belief, Genentech and City of Hope are co-assignees of the Cabilly II patent.

JURISDICTION AND VENUE

9. This action arises under the Declaratory Judgment Act of 1934 (28 U.S.C. §§ 2201-2201), Title 28 of the United States Code, for the purposes of determining an actual and justiciable controversy between the parties, and the patent laws of the United States, Title 35 of the United States Code. This Court has subject matter jurisdiction pursuant to 28 U.S.C. §§ 1331 and 1338(a) (2006).

10. This Court has personal jurisdiction over Genentech based on its principal place of business in California. This Court has personal jurisdiction over City of Hope based on its organization under the laws of the State of California and because its principal place of operation is in California.

11. Venue is proper in this District pursuant to 28 U.S.C. § 1391 (2006) because both Defendants reside in this District and a substantial part of the events or omissions giving rise to the claims occurred in this District.

INTRADISTRICT ASSIGNMENT

12. A substantial part of the events or omissions giving rise to the claims occurred in the San Francisco Division.

THE CABILLY PATENTS

13. On April 8, 1983, Shmuel Cabilly, Herbert Heyneker, William Holmes, Arthur Riggs, and Ronald Wetzel (the "Cabilly Applicants") filed a patent application in the United States Patent and Trademark Office ("PTO") that issued on March 28, 1989, as U.S. Patent 4,816,567 (the "Cabilly I patent"). The Cabilly Applicants assigned their rights to Genentech and the City of Hope.

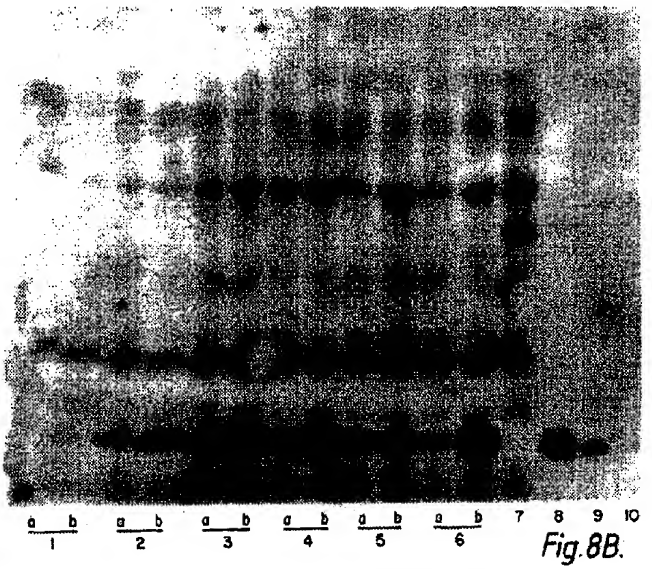
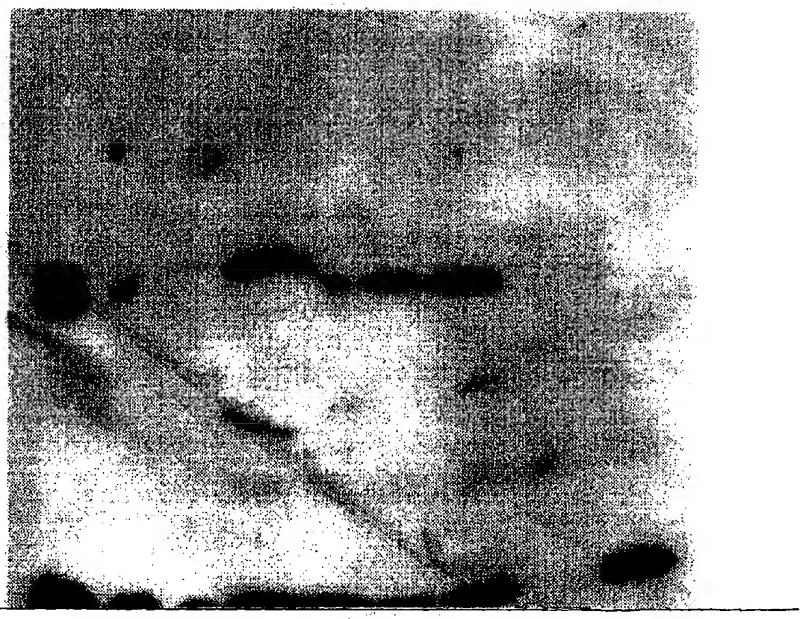


Fig. 8B.



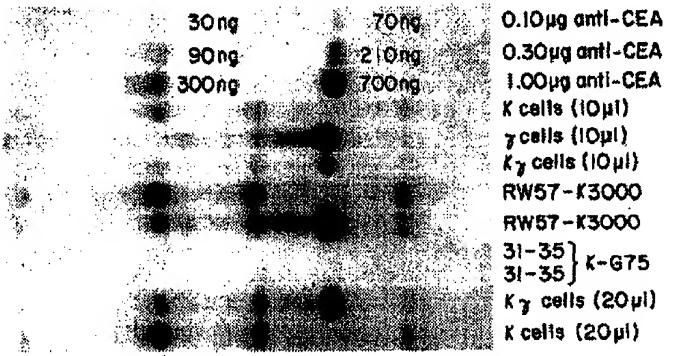

30ng
90ng
300ng
70ng
210ng
700ng
0.10µg anti-CEA
0.30µg anti-CEA
1.00µg anti-CEA
K cells (10µl)
γ cells (10µl)
Kγ cells (10µl)
RW57-K3000
RW57-K3000
31-35
31-35
K-G75
Kγ cells (20µl)
K cells (20µl)

Fig. 9.

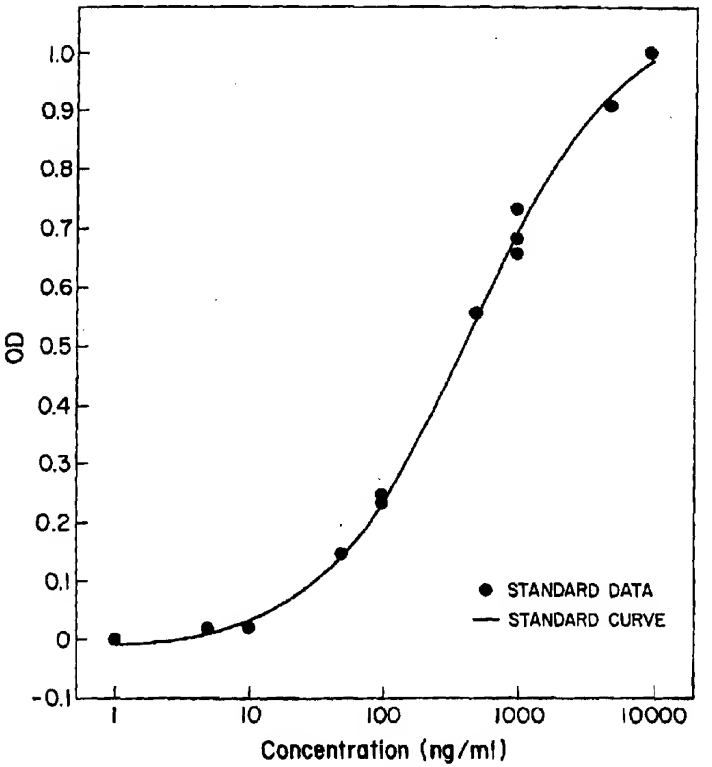

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
OD
1
10
100
1000
10000
Concentration (ng/ml)
STANDARD DATA
STANDARD CURVE

Fig. 10.

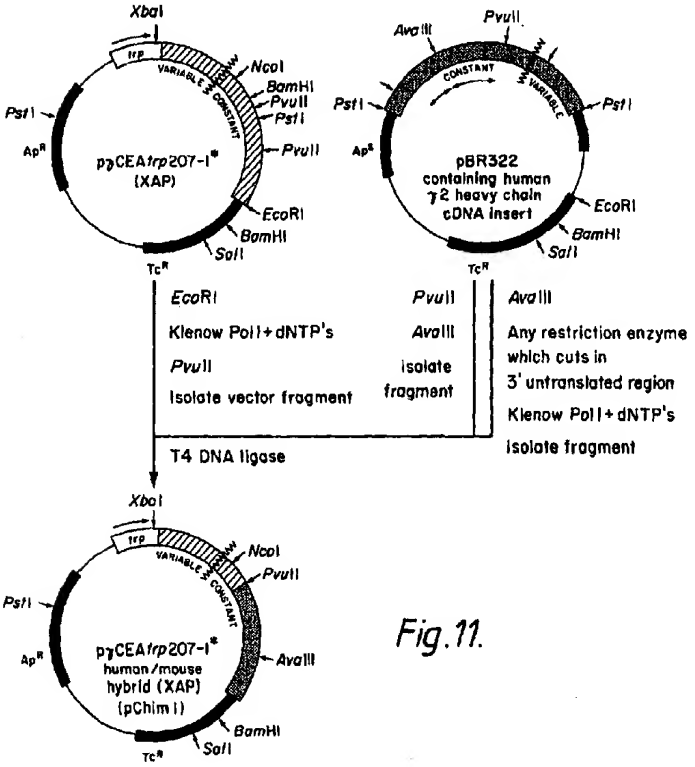

XbaI
trp
VARIABLE
CONSTANT
NcoI
BamHI
PvuII
PstI
PvuII
EcoRI
BamHI
SalI
PstI
Ap^R
Tc^R
pγCEAtrp207-1*
(XAP)
AvaIII
PvuII
CONSTANT
3' VARIABLE
PstI
EcoRI
BamHI
SalI
pBR322
containing human
γ2 heavy chain
cDNA insert
EcoRI
Klenow Pol I + dNTP's
PvuII
Isolate vector fragment
PvuII
AvaIII
isolate
fragment
AvaIII
Any restriction enzyme
which cuts in
3' untranslated region
Klenow Pol I + dNTP's
isolate fragment
T4 DNA ligase
pγCEAtrp207-1*
human/mouse
hybrid (XAP)
(pChim1)

Fig. 11.

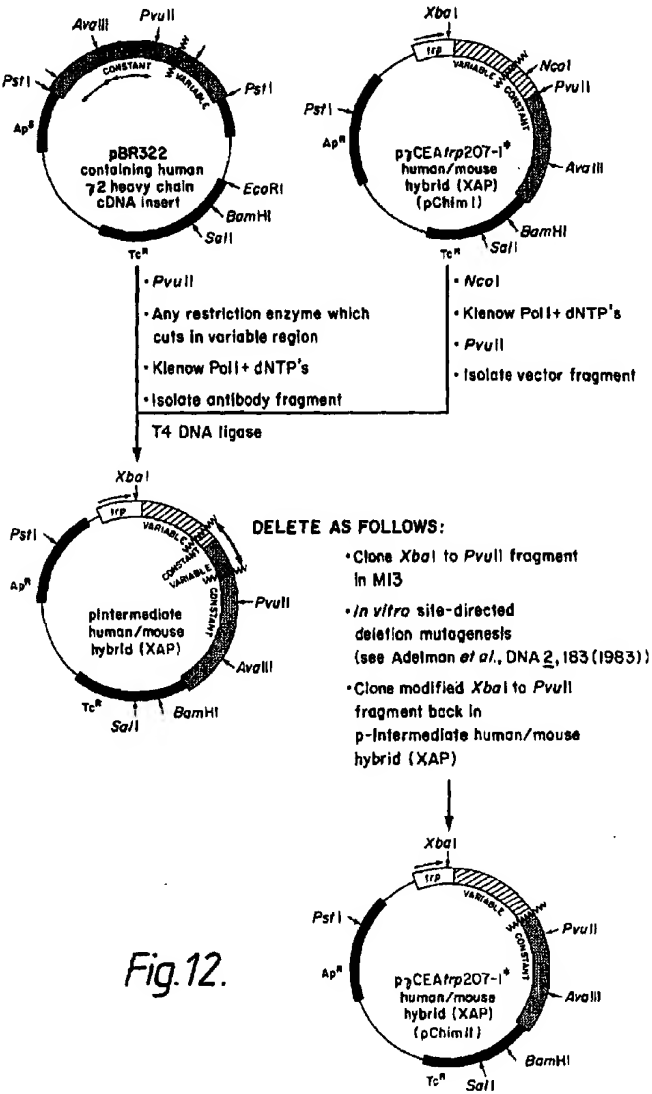

AvaIII
PvuII
PstI
CONSTANT
VARIABLE
PstI
Ap^R
pBR322
containing human
γ2 heavy chain
cDNA insert
EcoRI
BamHI
SalI
Tc^R
XbaI
trp
VARIABLE
NcoI
PvuII
PstI
Ap^R
pγCEAtrp207-1*
human/mouse
hybrid (XAP)
(pChim1)
AvaIII
BamHI
Tc^R
SalI
• PvuII
• Any restriction enzyme which cuts in variable region
• Klenow Pol I + dNTP's
• Isolate antibody fragment
• NcoI
• Klenow Pol I + dNTP's
• PvuII
• Isolate vector fragment
T4 DNA ligase
XbaI
trp
VARIABLE
CONSTANT
PstI
Ap^R
pIntermediate
human/mouse
hybrid (XAP)
PvuII
AvaIII
Tc^R
SalI
BamHI
DELETE AS FOLLOWS:
• Clone XbaI to PvuII fragment in MI3
• In vitro site-directed deletion mutagenesis (see Adelman et al., DNA 2, 183 (1983))
• Clone modified XbaI to PvuII fragment back in p-Intermediate human/mouse hybrid (XAP)
XbaI
trp
VARIABLE
CONSTANT
PstI
Ap^R
pγCEAtrp207-1*
human/mouse
hybrid (XAP)
(pChim11)
PvuII
AvaIII
BamHI
Tc^R
SalI

Fig. 12.

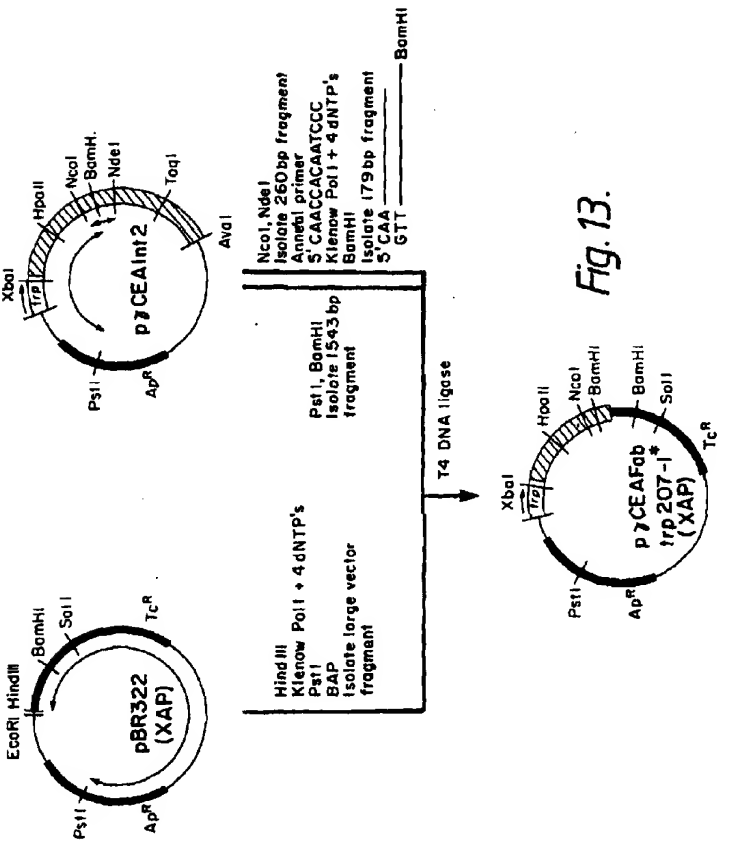

pBR322 (XAP)
EcoRI HindIII
BamHI
SalI
TcR
PstI
ApR
HindIII
Klenow Pol I + 4 dNTP's
PstI
BAP
Isolate large vector fragment
PstI, BamHI
Isolate 1543 bp fragment
NcoI, NdeI
Isolate 260bp fragment
Anneal primer
5' CAACCACAATCCC
Klenow Pol I + 4 dNTP's
BamHI
Isolate 179bp fragment
5'CAA
GTT
BamHI
T4 DNA ligase
pγCEAInt2
XbaI
trp
HpaII
NcoI
BamH.
NdeI
TaqI
AvaI
PstI
ApR
pγCEAFab trp207-1* (XAP)
XbaI
trp
HpaII
NcoI
BamHI
BamHI
SalI
TcR
PstI
ApR

Fig. 13.

METHODS OF PRODUCING IMMUNOGLOBULINS, VECTORS AND TRANSFORMED HOST CELLS FOR USE THEREIN

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 06/483,457, filed Apr. 8, 1983, now U.S. Pat. No. 4,816,567, issued Mar. 28, 1989.

BACKGROUND OF THE INVENTION

This invention relates to the field of immunoglobulin production and to modification of naturally occurring immunoglobulin amino acid sequences. Specifically, the invention relates to using recombinant techniques to produce both immunoglobulins which are analogous to those normally found in vertebrate systems and to take advantage of these gene modification techniques to construct chimeric or other modified forms.

A. Immunoglobulins and Antibodies

Antibodies are specific immunoglobulin polypeptides produced by the vertebrate immune system in response to challenge by foreign proteins, glycoproteins, cells, or other antigenic foreign substances. The sequence of events which permits the organism to overcome invasion by foreign cells or to rid the system of foreign substances is at least partially understood. An important part of this process is the manufacture of antibodies which bind specifically to a particular foreign substance. The binding specificity of such polypeptides to a particular antigen is highly refined, and the multitude of specificities capable of being generated by the individual vertebrate is remarkable in its complexity and variability. Thousands of antigens are capable of eliciting responses, each almost exclusively directed to the particular antigen which elicited it.

Immunoglobulins include both antibodies, as above described, and analogous protein substances which lack antigen specificity. The latter are produced at low levels by the lymph system and in increased levels by myelomas.

A.1 Source and Utility

Two major sources of vertebrate antibodies are presently utilized—generation in situ by the mammalian B lymphocytes and in cell culture by B-cell hybrids. Antibodies are made in situ as a result of the differentiation of immature B lymphocytes into plasma cells, which occurs in response to stimulation by specific antigens. In the undifferentiated B cell, the portions of DNA coding for the various regions on the immunoglobulin chains are separated in the genomic DNA. The sequences are reassembled sequentially prior to transcription. A review of this process has been given by Gough, *Trends in Biochem Sci*, 6: 203 (1981). The resulting rearranged genome is capable of expression in the mature B lymphocyte to produce the desired antibody. Even when only a single antigen is introduced into the sphere of the immune system for a particular mammal, however, a uniform population of antibodies does not result. The in situ immune response to any particular antigen is defined by the mosaic of responses to the various determinants which are present on the antigen. Each subset of homologous antibody is contributed by a single population of B-cells—hence in situ generation of antibodies is "polyclonal".

This limited but inherent heterogeneity has been overcome in numerous particular cases by use of hybridoma technology to create "monoclonal" antibodies (Kohler, et al., *Eur. J. Immunol.*, 6: 511 (1976)). In this process, splenocytes

or lymphocytes from a mammal which has been injected with antigen are fused with a tumor cell line, thus producing hybrid cells or "hybridomas" which are both immortal and capable of producing the genetically coded antibody of the B cell. The hybrids thus formed we segregated into single genetic strains by selection, dilution, and regrowth, and each strain thus represents a single genetic line. They therefore produce immunoreactive antibodies against a desired antigen which are assured to be homogenous, and which antibodies, referencing their pure genetic parentage, are called "monoclonal". Hybridoma technology has to this time been focused largely on the fusion of murine lines, but human-human hybridomas (Olsson, L. et al., *Proc. Natl. Acad. Sci. (USA)*, 77: 5429 (1980)); human-murine hybridomas (Schlom, J., et al. (ibid) 77: 6841 (1980)) and several other xenogenic hybrid combinations have been prepared as well. Alternatively, primary, antibody producing, B cells have been immortalized in vitro by transformation with viral DNA.

Polyclonal, or, much more preferably monoclonal, antibodies have a variety of useful properties similar to those of the present invention. For example, they can be used as specific immunoprecipitating reagents to detect the presence of the antigen which elicited the initial processing of the B cell genome by coupling this antigen-antibody reaction with suitable detection techniques such as labeling with radioisotopes or with enzymes capable of assay (RIA, EMIT, and ELISA). Antibodies are thus the foundation of immuno diagnostic tests for many antigenic substances. In another important use, antibodies can be directly injected into subjects suffering from an attack by a substance or organism containing the antigen in question to combat this attack. This process is currently in its experimental stages, but its potential is clearly seen. Third, whole body diagnosis and treatment is made possible because injected antibodies are directed to specific target disease tissues, and thus can be used either to determine the presence of the disease by carrying with them a suitable label, or to attack the diseased tissue by carrying a suitable drug.

Monoclonal antibodies produced by hybridomas, while theoretically effective as suggested above and clearly preferable to polyclonal antibodies because of their specificity, suffer from certain disadvantages. First, they tend to be contaminated with other proteins and cellular materials of hybridoma, (and, therefore, mammalian) origin. These cells contain additional materials, notably nucleic acid fragments, but protein fragments as well, which are capable of enhancing, causing, or mediating carcinogic responses. Second, hybridoma lines producing monoclonal antibodies tend to be unstable and may alter the structure of antibody produced or stop producing antibody altogether (Kohler, G., et al. *Proc. Natl. Acad. Sci (USA)* 77: 2197 (1980); Morrison, S. L., *J. Immunol.* 123: 793 (1979)). The cell line genome appears to alter itself in response to stimuli whose nature is not currently known, and this alteration may result in production of incorrect sequences. Third, both hybridoma and B cells inevitably produce certain antibodies in glycosylated form (Melchers, F., *Biochemistry*, 10: 653 (1971)) which, under some circumstances, may be undesirable. Fourth, production of both monoclonal and polyclonal antibodies is relatively expensive. Fifth, and perhaps most important, production by current techniques (either by hybridoma or by B cell response) does not permit manipulation of the genome so as to produce antibodies with more effective design components than those normally elicited in response to antigens from the mature B cell in situ. The antibodies of the present invention do not suffer from the

foregoing drawbacks, and, furthermore, offer the opportunity to provide molecules of superior design.

Even those immunoglobulins which lack the specificity of antibodies are useful, although over a smaller spectrum of potential uses than the antibodies themselves. In presently understood applications, such immunoglobulins are helpful in proteins replacement therapy for globulin related anemia. In this context, an inability to bind to antigen is in fact helpful, as the therapeutic value of these proteins would be impaired by such functionality. At present, such non-specific antibodies are derivable in quantity only from myeloma cell cultures suitably induced. The present invention offers an alternative, more economical source. It also offers the opportunity of canceling out specificity by manipulating the four chains of the tetramer separately.

A.2 General Structure Characteristics

The basic immunoglobin structural unit in vertebrate systems is now well understood (Edelman, G. M., *Ann. N.Y. Acad. Sci.*, 190: 5 (1971)). The units are composed of two identical light polypeptide chains of molecular weight approximately 23,000 daltons, and two identical heavy chains of molecular weight 53,000–70,000. The four chains are joined by disulfide bonds in a "Y" configuration wherein the light chains bracket-the heavy-chains starting at the mouth of the Y and continuing through the divergent region as shown in FIG. 1. The "branch" portion, as there indicated, is designated the Fab region. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, with some subclasses among them, and the nature of this chain, as it has a long constant region, determines the "class"of the antibody as IgG, IgM, IgA, IgD, or IgE. Light chains are classified-as either kappa or lambda. Each heavy chain class can be prepared with either kappa or lambda light chain. The light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages when the immunoglobulins are generated either by hybridomas or by B cells. However, if non-covalent association of the chains can be effected in the correct geometry, the aggregate will still be capable of reaction with antigen, or of utility as a protein supplement as a non-specific immunoglobulin.

The amino acid sequence runs from the N-terminal end at the top of the Y to the C-terminal end at the bottom of each chain. At the N-terminal end is a variable region which is specific for the antigen which elicited it, and is approximately 100 amino acids in length, there being slight variations between light and heavy chain and from antibody to antibody. The variable region is linked in each chain to a constant region which extends the remaining length of the chain. Linkage is seen, at the genomic level, as occurring through a linking sequence known currently as the "J" region in the light chain gene, which encodes about 12 amino acids, and as a combination of "D" region and "J" region in the heavy chain gene, which together encode approximately 25 amino acids.

The remaining portions of the chain are referred to as constant regions and within a particular class do not to vary with the specificity of the antibody (i.e., the antigen eliciting it).

As stated above, there are five known major classes of constant regions which determine the class of the immunoglobulin molecule (IgG, IgM, IgA, IgD, and IgE corresponding to γ, μ, α, δ, and ε heavy chain constant regions). The constant region or class determines subsequent effector function of the antibody, including activation of complement (Kabat, E. A., *Structural Concepts in Immunology and Immunochemistry*, 2nd Ed., p. 413–436, Holt, Rinehart Winston (1976)), and other cellular responses (Andrews, D. W., et al., *Clinical Immunobiology* pp 1–18, W. B. Sanders (1980); Kohl, S., et al., *Immunology*, 48: 187 (1983)); while the variable region determines the antigen with which it will react.

B. Recombinant DNA Technology

Recombinant DNA technology has reached sufficient sophistication that it includes a repertoire of techniques for cloning and expression of gene sequences. Various DNA sequences can be recombined with some facility, creating new DNA entities capable of producing heterologous protein product in transformed microbes and cell cultures. The general means and methods for the in vitro ligation of various blunt ended or "sticky" ended fragments of DNA, for producing expression vectors, and for transforming organisms are now in hand.

DNA recombination of the essential elements (i.e., an origin of replication, one or more phenotypic selection characteristics, expression control sequence, heterologous gene insert and remainder vector) generally is performed outside the host cell. The resulting recombinant replicable expression vector, or plasmid, is introduced into cells by transformation and large quantities of the recombinant vehicle is obtained by growing the transformants. Where the gene is properly inserted with reference to portions which govern the transcription and translation of the encoded DNA message, the resulting expression vector is useful to produce the polypeptide sequence for which the inserted gene codes, a process referred to as "expression." The resulting product may be obtained by lysis, if necessary, of the host cell and recovery of the product by appropriate purifications from other proteins.

In practice, the use of recombinant DNA technology can express entirely heterologous polypeptides—so-called direct expression—or alternatively may express a heterologous polypeptide fused to a portion of the amino acid sequence of a homologous polypeptide. In the latter cases, the intended bioactive product is sometimes rendered bioinactive within the fused, homologous/heterologous polypeptide until it is cleaved in an extracellular environment.

The art of maintaining cell or tissue cultures as well as microbial systems for studying genetics and cell physiology is well established. Means and methods are available for maintaining permanent cell lines, prepared by successive serial transfers from isolated cells. For use in research, such cell lines are maintained on a solid support in liquid medium, or by growth in suspension containing support nutriments. Scale-up for large preparations seems to pose only mechanical problems.

SUMMARY OF THE INVENTION

The invention relates to antibodies and to non-specific immunoglobulins (NSIs) formed by recombinant techniques using suitable host cell cultures. These antibodies and NSIs can be readily prepared in pure "monoclonal" form. They can be manipulated at the genomic level to produce chimeras of variants which draw their homology from species which differ from each other. They can also be manipulated at the protein level, since all four chains do not need to be produced by the same cell. Thus, there are a number of "types" of immunoglobulins encompassed by the invention.

First, immunoglobulins, particularly antibodies, are produced using recombinant techniques which mimic the amino acid sequence of naturally occurring antibodies produced by either mammalian B cells in situ, or by B cells fused with suitable immortalizing tumor lines, i.e., hybridomas.

Second, the methods of this invention produce, and the invention is directed to, immunoglobulins which comprise polypeptides not hitherto found associated with each other in nature. Such reassembly is particularly useful in producing "hybrid" antibodies capable of binding more than one antigen; and in producing "composite" immunoglobulins wherein heavy and light chains of different origins essentially damp out specificity. Third, by genetic manipulation, "chimeric" antibodies can be formed wherein, for example, the variable regions correspond to the amino acid sequence from one mammalian model system, whereas the constant region mimics the amino acid sequence of another. Again, the derivation of these two mimicked sequences may be from different species. Fourth, also by genetic manipulation, "altered" antibodies with improved specificity and other characteristics can be formed.

Two other types of immunoglobulin-like moieties may be produced: "univalent" antibodies, which are useful as homing carriers to target tissues, and "Fab proteins" which include only the "Fab" region of an immunoglobulin molecule i.e., the branches of the "Y". These univalent antibodies and Fab fragments may also be "mammalian" i.e., mimic mammalian amino acid sequences; novel assemblies of mammalian chains, or chimeric, where for example, the constant and variable sequence patterns may be of different origin. Finally, either the light chain or heavy chain alone, or portions, thereof, produced by recombinant techniques are included in the invention and may be mammalian or chimeric.

In other aspects, the invention is directed to DNA which encodes the aforementioned NSIs, antibodies, and portions thereof, as well as expression vectors or plasmids capable of effecting the production of such immunoglobulins in suitable host cells. It includes the host cells and cell cultures which result from transformation with these vectors. Finally, the invention is directed to methods of producing these NSIs and antibodies, and the DNA sequences, plasmids, and transformed cells intermediate to them.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a representation of the general structure of immunoglobulins.

FIGS. 2A–B shows the detailed sequence of the cDNA insert of pK17G4 which encodes kappa anti CEA chain.

FIG. 3 shows the coding sequence of the fragment shown in FIG. 2, along with the corresponding amino acid sequence.

FIGS. 4A–C shows the combined detailed sequence of the cDNA inserts of py298 and py11 which encode gamma anti CEA chain.

FIGS. 5A–B shows the corresponding amino acid sequence encoded by the fragment in FIG. 4.

FIGS. 6 and 7 outline the construction of expression vectors for kappa and gamma anti-CEA chains respectively.

FIGS. 8A, 8B, and 8C show the results of sizing gels run on, extracts of *E. coli* expressing the genes for gamma chain, kappa chain, and both kappa and gamma chains respectively.

FIG. 9 shows the results of western blots of extracts of cells transformed as those in FIGS. 8.

FIG. 10 shows a standard curve for ELISA assay of anti CEA activity.

FIGS. 11 and 12 show the construction of a plasmid for expression of the gene encoding a chimeric heavy chain.

FIG. 13 shows the construction of a plasmid for expression of the gene encoding the Fab region of heavy chain.

DETAILED DESCRIPTION

A. Definitions

As used herein, "antibodies" refers to tetramers or aggregates thereof which have specific immunoreactive activity, comprising light and heavy chains usually aggregated in the "Y" configuration of FIG. 1, with or without covalent linkage between them; "immunoglobulins" refers to such assemblies whether or not specific immunoreactive activity is a property. "Non-specific immunoglobulin" ("NSI") means those immunoglobulins which do not possess specificity—i.e., those which are not antibodies.

"Mammalian antibodies" refers to antibodies wherein the amino acid sequences of the chains are homologous with those sequences found in antibodies produced by mammalian systems, either in situ, or in hybridomas. These antibodies mimic antibodies which are otherwise capable of being generated, although in impure form, in these traditional systems.

"Hybrid antibodies" refers to antibodies wherein chains are separately homologous with referenced mammalian antibody chains and represent novel assemblies of them, so that two different antigens are precipitable by the tetramer. In hybrid antibodies, one pair of heavy and light chain is homologous to antibodies raised against one antigen, while the other pair of heavy and light chain is homologous to those raised against another antigen. This results in the property of "divalence" i.e., ability to bind two antigens simultaneously. Such hybrids may, of course, also be formed using chimeric chains, as set forth below.

"Composite" immunoglobulins means those wherein the heavy and light chains mimic those of different species origins or specificities, and the resultant is thus likely to be a non-specific immunoglobulin (NSI), i.e.—lacking in antibody character.

"Chimeric antibodies" refers to those antibodies wherein one portion of each of the amino acid sequences of heavy and light chains is homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular class, while the remaining segment of the chains is homologous to corresponding sequences in another. Typically, in these chimeric antibodies, the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals while the constant portions are homologous to the sequences in antibodies derived from another. One clear advantage to such chimeric forms is that, for example, the variable regions can conveniently be derived from presently known sources using readily available hybridomas or B cells from non human host organisms in combination with constant regions derived from, for example, human cell preparations. While the variable region has the advantage of ease of preparation, and the specificity is not affected by its source, the constant region being human, is less likely to elicit an immune response from a human subject when the antibodies are injected than would the constant region from a non-human source.

However, the definition is not limited to this particular example. It includes any antibody in which either or both of the heavy or light chains are composed of combinations of sequences mimicking the sequences in antibodies of different sources, whether these sources be differing classes, differing antigen responses, or differing species of origin and whether or not the fusion point is At the variable/constant boundary. Thus, it is possible to produce antibodies in which neither the constant nor the variable region mimic known antibody sequences. It then becomes possible, for example, to construct antibodies whose variable region has a higher

Patent Interference

14. At the time the Cabilly I patent issued, the Cabilly Applicants had a continuation application (the "Cabilly II application") pending in the PTO. The Cabilly Applicants copied claims from U.S. Patent 4,816,397 (the "Boss patent") in order to provoke the PTO Board of Patent Appeals and Interferences to initiate an interference proceeding to determine whether the Boss patentees or the Cabilly Applicants were entitled to priority for the inventions claimed in the Boss patent.

15. In February 1991, the PTO Board declared a patent interference between the pending Cabilly II application and the Boss patent on the ground that both the Boss patentees and the Cabilly Applicants claimed the same purported invention. After seven years of adversarial proceedings in the PTO, in August 1998, the PTO Board found that the Boss patentees were entitled to priority over the Cabilly Applicants. *See Cabilly v. Boss*, 55 U.S.P.Q.2d 1238 (B.P.A.I. 1998). The PTO Board concluded that the Cabilly Applicants had failed to establish conception or reduction to practice of the claimed inventions prior to March 25, 1983 – the filing date of the Boss patent. According to the PTO Board, "there is no evidence that immunoglobulins, multiple chain proteins, had been produced by recombinant DNA techniques from a single host cell prior to March 25, 1983." Moreover, "the evidence indicates that Cabilly et al. had but a **hope or wish to produce active antibodies in bacteria**; and, there is no supporting evidence to establish the development of the means to accomplish that result or evidence of a disclosure to a third party of complete conception." (emphasis added). The Final Decision therefore indicated that the Cabilly Applicants were "not entitled to a patent."

16. In October 1998, Genentech filed an action in this District under 35 U.S.C. § 146 against the owner of the Boss patent, Celltech Therapeutics Ltd. ("Celltech"), to appeal the decision of the PTO Board awarding priority to the Boss patent. *Genentech, Inc. v. Celltech Therapeutics Ltd.*, Case No. C98-3926 (N.D. Cal.). In March 2001, the parties to that action filed a notice of settlement and joint request for entry of settlement instruments. As part of their settlement agreement, the parties asked the district court to find that, contrary to the PTO Board's prior decision, Genentech's Cabilly Applicants were entitled to priority. On information and belief, as part of the Genentech-Celltech agreement, Celltech obtained certain rights relating to the Cabilly II

specific affinity for a particular antigen, or whose constant region can elicit enhanced complement fixation or to make other improvements in properties possessed by a particular constant region.

"Altered antibodies" means antibodies wherein the amino acid sequence has been varied from that of a mammalian or other vertebrate antibody. Because of the relevance of recombinant DNA techniques to this invention, one need not be confined to the sequences of amino acids found in natural antibodies; antibodies can be redesigned to obtain desired characteristics. The possible variations are many and range from the changing of just one or a few amino acids to the complete redesign of, for example, the constant region. Changes in the constant region will, in general, be made in order to improve the cellular process characteristics, such as complement fixation, interaction with membranes, and other effector functions. Changes in the variable region will be made in order to improve the antigen binding characteristics. The antibody can also be engineered so as to aid the specific delivery of a toxic agent according to the "magic bullet" concept. Alterations, can be made by standard recombinant techniques and also by oligonucleotide-directed mutagenesis techniques (Dalbadie-McFarland, et al *Proc. Natl. Acad. Sci.* (*USA*), 79:6.409 (1982)).

"Univalent antibodies" refers to aggregations which comprise a heavy chain/light chain dimer bound to the Fc (or stem) region of a second heavy chain. Such antibodies are specific for antigen but have the additional desirable property of targeting tissues with specific antigenic surfaces, without causing its antigenic effectiveness to be impaired— i.e., there is no antigenic modulation. This phenomenon and the property of univalent antibodies in this regard is set forth in Glennie, M. J., et al., *Nature*, 295: 712 (1982). Univalent antibodies have heretofore been formed by proteolysis.

"Fab" region refers to those portions of the chains which are roughly equivalent, or analogous, to the sequences which comprise the Y branch portions of the heavy chain and to the light chain in its entirety, and which collectively (in aggregates) have been shown to exhibit antibody activity. "Fab protein", which protein is one of the aspects of the invention, includes aggregates of one heavy and one light chain (commonly known as Fab'), as well as tetramers which correspond to the two branch segments of the antibody Y, (commonly known as $F(ab)_2$), whether any of the above are covalently or non-covalently aggregated, so long as the aggregation is capable of selectively reacting with a particular antigen or antigen family. Fab antibodies have, as have univalent ones, been formed heretofore by proteolysis, and share the property of not eliciting antigen modulation on target tissues. However, as they lack the "effector" Fc: portion they cannot effect, for example, lysis of the target cell by macrophages.

"Fab protein" has similar subsets according to the definition of the present invention as does the general term "antibodies" or "immunoglobulins". Thus, "mammalian" Fab protein, "hybrid" Fab protein "chimeric" Fab and "altered" Fab protein are defined analogously to the corresponding definitions set forth in the previous paragraphs for the various types of antibodies.

Individual heavy or light chains may of course be "mammalian", "chimeric" or "altered" in accordance with the above. As will become apparent from the detailed description of the invention, it is possible, using the techniques disclosed to prepare other combinations of the four-peptide chain aggregates, besides those specifically defined, such as hybrid antibodies containing chimeric light and mammalian heavy chains, hybrid Fab proteins containing chimeric Fab proteins of heavy chains associated with mammalian light chains, and so forth.

"Expression vector" includes vectors which are capable of expressing DNA sequences contained therein, i.e., the coding sequences are operably linked to other sequences capable of effecting their expression. It is implied, although not always explicitly stated, that these expression vectors must be replicable in the host organisms either as episomes or as an integral part of the chromosomal DNA. Clearly a lack of replicability would render them effectively inoperable. A useful, but not a necessary, element of an effective expression vector is a marker encoding sequence—i.e. a sequence encoding a protein which results in a phenotypic property (e.g. tetracycline resistance) of the cells containing the protein which permits those cells to be readily identified. In sum, "expression vector" is given a functional definition, and any DNA sequence which is capable of effecting expression of a specified contained DNA code is included in this term, as it is applied to the specified sequence. As at present, such vectors are frequently in the form of plasmids, thus "plasmid" and "expression vector" are often used interchangeably. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which may, from time to time become known in the art.

"Recombinant host cells" refers to cells which have been transformed with vectors constructed using recombinant DNA techniques. As defined herein, the antibody or modification thereof produced by a recombinant host cell is by virtue of this transformation, rather than in such lesser amounts, or more commonly, in such less than detectable amounts, as would be produced by the untransformed host.

In descriptions of processes for isolation of antibodies from recombinant hosts, the terms "cell" and "cell culture" are used interchangeably to denote the source of antibody unless it is clearly specified otherwise. In other words, recovery of antibody from the "cells" may mean either from spun down whole cells, or from the cell culture containing both the medium and the suspended cells.

B. Host Cell Cultures and Vectors

The vectors and methods disclosed herein are suitable for use in host cells over a wide range of prokaryotic and eukaryotic organisms.

In general, of course, prokaryotes are preferred for cloning of DNA sequences in constructing the vectors useful in the invention. For example, *E. coli* K12 strain 294 (ATCC No. 31446) is particularly useful. Other microbial strains which may be used include *E. coli* strains such as *E. coli* B, and *E. coli* X1776 (ATTC No. 31537). These examples are, of course, intended to be illustrative rather than limiting.

Prokaryotes may also be used for expression. The aforementioned strains, as well as *E. coli* W3110 (F^-, λ^-, prototrophic, ATTC No. 27325), bacilli such as *Bacillus subtilus*, and other enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcesans*, and various Pseudomonas species may be used.

In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species (Bolivar, et al., *Gene* 2: 95 (1977)). PBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR322 plasmid, or other microbial

plasmid must also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of its own proteins. Those promoters most commonly used in recombinant DNA construction include the β-lactamase (penicillinase) and lactose promoter systems Chang et al, *Nature* 275: 615 (1978); Itakura, et al, *Science* 198: 1056 (1977); (Goeddel et al, *Nature* 281: 544 (1979)) and a tryptophan (trp) promoter system (Goeddel, et al, *Nucleic Acids Res.*, 8: 4057 (1980); EPO Appl Publ No. 0036776). While these are the most commonly used, other microbial promoters have been discovered and utilized., and details concerning their nucleotide sequences have been published, enabling a skilled worker to ligate them functionally with plasmid vectors (Siebenlist, et al, Cell 20: 269 (1980)).

In addition to prokaryates, eukaryotic microbes, such as yeast cultures may also be used. *Saccharomyces cerevisiae*, or common baker's yeast is the most commonly used among eukaryotic microorganisms, although a number of other strains are commonly available. For expression in Saccharomyces, the plasmid YRp7, for example, (Stinchcomb et al, *Nature*, 282: 39 (1979); Kingsman et al, *Gene*, 7: 141 (1979); Tschemper, et al, *Gene*, 10: 157 (1980)) is commonly used. This plasmid already contains the trp1 gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, *Genetics*, 85: 12 (1977)). The presence of the trp1 lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable promoting sequences in yeast vectors include the promoters for 3-phosphoglycerate kinase (Hitzeman, et al., *J. Biol. Chem.*, 255: 2073 (1980)) or other glycolytic enzymes (Hess, et al, *J. Adv. Enzyme Reg.*, 7: 149 (1968); Holland, et al, *Biochemistry*, 17: 4900 (1978)), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. In constructing suitable expression plasmids, the termination sequences associated with these genes are also ligated into the expression vector 3' of the sequence desired to be expressed to provide polyadenylation of the mRNA and termination. Other promoters, which have the additional advantage of transcription controlled by growth conditions are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and the aforementioned glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization (Holland, ibid.). Any plasmid vector containing yeast-compatible promoter, origin of replication and termination sequences is suitable.

In addition to microorganisms cultures of cells derived from multicellular organisms may also be used as hosts. In principle, any such cell culture is workable, whether from vertebrate or invertebrate culture. However interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years (*Tissue Culture*, Academic Press, Kruse and Patterson, editors (1973)). Examples of such useful host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and W138, BHK, COS-7 and MDCK cell lines. Expression vectors for such cells ordinarily include (if necessary) an origin of replication, a promoter located in front of the gene to be expressed, along with any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional terminator sequences.

For use in mammalian cells, the control functions on the expression vectors are often provided by viral material. For example, commonly used promoters are derived from polyoma, Adenovirus 2, and most frequently Simian Virus 40 (SV40). The early and late promoters of SV40 virus are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers, et al, *Nature*, 273: 113 (1978)) incorporated herein by reference. Smaller or larger SV40 fragments may also be used, provided there is included the approximately 250 bp sequence extending from the Hind III site toward the Bgl I site located in the viral origin of replication. Further, it is also possible, and often desirable, to utilize promoter or control sequences normally associated with the desired gene sequence, provided such control sequences are compatible with the host cell systems.

An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral (e.g. Polyoma, Adeno, VSV, BPV, etc.) source, or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter is often sufficient.

It will be understood that this invention, although described herein in terms of a preferred embodiment, should not be construed as limited to those host cells, vectors and expression systems exemplified.

C. Methods Employed

C.1 Transformation

If cells without formidable cell wall barriers are used as host cells, transfection is carried out by the calcium phosphate precipitation method as described by Graham and Van der Eb, *Virology*, 52: 546 (1978). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used.

If prokaryotic cells or cells which contain substantial cell wall constructions are used, the preferred method of transfection is calcium treatment using calcium chloride as described by Cohen, F. N. et al *Proc. Natl. Acad. Sci.* (*USA*) 69: 2110 (1972).

C.2 Vector Construction

Construction of suitable vectors containing the desired coding and control sequences employ standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to form the plasmids required. The methods employed are not dependent on the DNA source, or intended host.

Cleavage is performed by treating with restriction enzyme (or enzymes) in suitable buffer. In general, about 1 μg plasmid or DNA fragments is used with about 1 unit of enzyme in about 20 μl of buffer solution. (Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer.) Incubation times of about 1 hour at 37° C. are workable. After incubations, protein is removed by extraction with phenol and chloroform, and the nucleic acid is recovered from the aqueous fraction by precipitation with ethanol.

If blunt ends are required, the preparation is treated for 15 minutes at 15° with 10 units of *E. coli* DNA Polymerase I (Klenow), phenol-chloroform extracted, and ethanol precipitated.

Size separation of the cleaved fragments is performed using 6 percent polyacrylamide gel described by Goeddel, D., et al, *Nucleic Acids Res.*, 8: 4057 (1980) incorporated herein by reference.

For ligation, approximately equimolar amounts of the desired components, suitably end tailored to provide correct matching are treated with about 10 units T4 DNA ligase per 0.5 μg DNA. (When cleaved vectors are used as components, it may be useful to prevent religation of the cleaved vector by pretreatment with bacterial alkaline phosphatase.)

In the examples described below correct ligations for plasmid construction are confirmed by transforming *E. coli* K12 strain 294 (ATCC 31446) with the ligation mixture. Successful transformants were selected by ampicillin or tetracycline resistance depending on the mode of plasmid construction. Plasmids from the transformants were then prepared, analyzed by restriction and/or sequenced by the method of Messing, et al, *Nucleic Acids Res.*, 9:309 (1981) or by the method of Maxam, et al, *Methods in Enzymology*, 65:499 (1980).

D. Outline of Procedures

D.1 Mammalian Antibodies

The first type of antibody which forms a part of this invention, and is prepared by the methods thereof, is "mammalian antibody"-one wherein the heavy and light chains mimic the amino acid sequences of an antibody otherwise produced by a mature mammalian B lymphocyte either in situ or when fused with an immortalized cell as part of a hybridoma culture. In outline, these antibodies are produced as follows:

Messenger RNA coding for heavy or light chain is isolated from a suitable source, either mature B cells or a hybridoma culture, employing standard techniques of RNA isolation, and the use of oligo-dT cellulose chromatography to segregate the poly-A mRNA. The poly-A mRNA may, further, be fractionated to obtain sequences of sufficient size to code for the amino acid sequences in the light or heavy chain of the desired antibody as the case may be.

A cDNA library is then prepared from the mixture of mRNA using a suitable primer, preferably a nucleic acid sequence which is characteristic of the desired cDNA. Such a primer may be hypothesized and synthesized based on the amino acid sequence of the antibody if the sequence is known. In the alternative cDNA from unfractionated poly-A mRNA from a cell line producing the desired antibody or poly-dT may also be used. The resulting cDNA is optionally size fractionated on polyacrylamide gel and then extended with, for example, dC residues for annealing with pBR322 or other suitable cloning vector which has been cleaved by a suitable restriction enzyme, such as Pst I, and extended with dG residues. Alternative means of forming cloning vectors containing the cDNA using other tails and other cloning vector remainder may, of course, also be used but the foregoing is a standard and preferable choice. A suitable host cell strain, typically *E. coli*, is transformed with the annealed cloning vectors, and the successful transformants identified by means of, for example, tetracycline resistance or other phenotypic characteristic residing on the cloning vector plasmid.

Successful transformants are picked and transferred to microtiter dishes or other support for further growth and preservation. Nitrocellulose filter imprints of these growing cultures are then probed with suitable nucleotide sequences containing bases known to be complementary to desired sequences in the cDNA. Several types of probe may be used, preferably synthetic single stranded DNA sequences labeled by kinasing with ATP^{32}. The cells fixed to the nitrocellulose filter are lysed, the DNA denatured, and then fixed before reaction with kinased probe. Clones which successfully hybridize are detected by contact with a photoplate, then plasmids from the growing colonies isolated and sequenced by means known in the art to verify that the desired portions of the gene are present.

The desired gene fragments are excised and tailored to assure appropriate reading frame with the control segments when inserted into suitable expression vectors. Typically, nucleotides are added to the 5' end to include a start signal and a suitably positioned restriction endonuclease site.

The tailored gene sequence is then positioned in a vector which contains a promoter in reading frame with the gene and compatible with the proposed host cell. A number of plasmids such as those described in U.S. patent application Ser. Nos. 307,473; 291,892; and 305,657 (EPO Publ. Nos. 0036776; 0048970 and 0051873) have been described which already contain the appropriate promoters, control sequences, ribosome binding sites, and transcription termination sites, as well as convenient markers.

In the present invention, the gene coding for the light chain and that coding for the heaving chain are recovered separately by the procedures outlined above. Thus then may be inserted into separate expression plasmids, or together in the same plasmid, so long as each is under suitable promoter and translation control.

The expression vectors constructed above are then used to transform suitable cells. The light and heavy chains may be transformed into separate cell cultures, either of the same or of differing species; separate plasmids for light and heavy chain may be used to co-transform a single cell culture, or, finally, a single expression plasmid containing both genes and capable of expressing the genes for both light and heavy chain may be transformed into a single cell culture.

Regardless of which of the three foregoing options is chosen, the cells are grown under conditions appropriate to the production of the desired protein. Such conditions are primarily mandated by the type of promoter and control systems used in the expression vector, rather than by the nature of the desired protein. The protein thus produced is then recovered from the cell culture by methods known in the art, but choice of which is necessarily dependent on the form in which the protein is expressed. For example, it is common for mature heterologous proteins expressed in *E. coli* to be deposited within the cells as insoluble particles which require cell lysis and solubilization in denaturant to permit recovery. On the other hand, proteins under proper synthesis circumstances, in yeast and bacterial strains, can be secreted into the medium (yeast and gram positive bacteria) or into the periplasmic space (gram negative bacteria) allowing recovery by less drastic procedures. Tissue culture cells as hosts also appear, in general, to permit reasonably facile recovery of heterologous proteins.

When heavy and light chain are coexpressed in the same host, the isolation procedure is designed so as to recover reconstituted antibody. This can be accomplished in vitro as described below, or might be possible in vivo in a microorganism which secretes the IgG chains out of the reducing environment of the cytoplasm. A more detailed description is given in D.2, below.

D.2 Chain Recombination Techniques

The ability of the method of the invention to produce heavy and light chains or portions thereof, in isolation from each other offers the opportunity to obtain unique and unprecedented assemblies of immunoglobulins, Fab regions, and univalent antibodies. Such preparations require the use of techniques to reassemble isolated chains. Such means are known in the art, and it is, thus, appropriate to review them here.

While single chain disulfide bond containing proteins have been reduced and reoxidized to regenerate in high yield

native structure and activity (Freedman, R. B., et al. In *Enzymology of Post Translational Modification of Proteins*, 1: 157–212 (1980) Academic Press, NY.), proteins which consist of discontinuous polypeptide chains held together by disulfide bonds are more difficult to reconstruct in vitro after reductive cleavage. Insulin, a cameo case, has received much experimental attention over the years, and can now be reconstructed so efficiently that an industrial process has been built around it (Chance, R. E., et al., In *Peptides: Proceedings of the Seventh Annual American Peptide Symposium* (Rich, D. H. and Gross. E., eds.) 721–728, Pierce Chemical Co., Rockford, Ill. (1981)).

Immunoglobulin has proved a more difficult problem than insulin. The tetramer is stabilized intra and intermolecularly by 15 or more disulfide bonds. It has been possible to recombine heavy and light chains, disrupted by cleavage of only the interchain disulfides, to regain antibody activity even without restoration of the inter-chain disulfides (Edelman, G. M., et al., *Proc. Natl. Acad. Sci. (USA)* 50: 753 (1963)). In addition, active fragments of IgG formed by proteolysis (Fab fragments of ~50,000 MW) can be split into their fully reduced heavy chain and light chain components and fairly efficiently reconstructed to give active antibody (Haber, E., *Proc. Natl. Acad. Sci. (USA)* 52: 1099 (1964); Whitney, P. L., et al., *Proc. Natl. Acad. Sci. (USA)* 53: 524 (1965)). Attempts to reconstitute active antibody from fully reduced native IgG have been largely unsuccessful, presumably due to insolubility of the reduced chains and of side products or intermediates in the refolding pathway (see discussion in Freedman, M. H., et al., *J. Biol. Chem.* 241: 5225 (1966)). If, however, the immunoglobulin is randomly modified by polyalanylation of its lysines before complete reduction, the separated chains have the ability to recover antigen-combining activity upon reoxidation (ibid).

A particularly suitable method for immunoglobulin reconstitution is derivable from the now classical insulin recombination studies, wherein starting material was prepared by oxidative sulfitolysis, thus generating thiol-labile S-sulfonate groups at all cysteines in the protein, non-reductively breaking disulfides (Chance et al. (supra)). Oxidative sulfitolysis is a mild disulfide cleavage reaction (Means, G. E., et al., *Chemical Modification of Proteins*, Holden-Day, San Francisco (1971)) which is sometimes more gentle than reduction, and which generates derivatives which are stable until exposed to mild reducing agent at which time disulfide reformation can occur via thiol-disulfide interchange. In the present invention the heavy and light chain S-sulfonates generated by oxidative sulfitolysis were reconstituted utilizing both air oxidation and thiol-disulfide interchange to drive disulfide bond formation. The general procedure is set forth in detail in U.S. Ser. No. 452,187, filed Dec. 22, 1982 (EPO Appln. No. 83.307840.5), incorporated herein by reference.

D.3 Variants Permitted by Recombinant Technology

Using the techniques described in paragraphs D.1 and D.2, additional operations which were utilized to gain efficient production of mammalian antibody can be varied in quite straightforward and simple ways to produce a great variety of modifications of this basic antibody form. These variations are inherent in the use of recombinant technology, which permits modification at a genetic level of amino acid sequences in normally encountered mammalian immunoglobulin chains, and the great power of this approach lies in its ability to achieve these variations, as well as in its potential for economic and specific production of desired scarce, and often contaminated, molecules. The variations also inhere in the ability to isolate production of individual chains, and thus create novel assemblies.

Briefly, since genetic manipulations permit reconstruction of genomic material in the process of construction of expression vectors, such reconstruction can be manipulated to produce new coding sequences for the components of "natural" antibodies or immunoglobulins. As discussed in further detail below, the coding sequence for a mammalian heavy chain may not be derived entirely from a single source or single species, but portions of a sequence can be recovered by the techniques described in D.1 from differing pools of mRNA, such as murine-murine hybridomas, human-murine hybridomas, or B cells differentiated in response to a series of antigen challenges. The desired portions of the sequences in each case can be recovered using the probe and analysis techniques described in D.1, and recombined in an expression vector using the same ligation procedures as would be employed for portions of the same model sequence. Such chimeric chains can be constructed of any desired length; hence, for example, a complete heavy chain can be constructed, or only sequence for the Fab region thereof.

The additional area of flexibility which arises from the use of recombinant techniques results from the power to produce heavy and light chains or fragments thereof in separate cultures or of unique combinations of heavy and light chain in the same culture, and to prevent reconstitution of the antibody or immunoglobulin aggregation until the suitable components are assembled. Thus, while normal antibody production results automatically in the formation of "mammalian antibodies" because the light and heavy chain portions are constructed in response to a particular determinant in the same cell, the methods of the present invention present the opportunity to assemble entirely new mixtures. Somewhat limited quantities of "hybrid" antibodies have been produced by "quadromas" i.e., fusions of two hybridoma cell cultures which permit random assemblies of the heavy and light chains so produced.

The present invention permits a more controlled assembly of desired chains, either by mixing the desired chains in vitro, or by transforming the same culture with the coding sequences for the desired chains.

D.4 Composite Immunoglobulins

The foregoing procedure, which describes in detail the recombinant production of mammalian antibodies is employed with some modifications to construct the remaining types of antibodies or NSIs encompassed by the present invention. To prepare the particular embodiment of composite non-specific immunoglobulin wherein the homology of the chains corresponds to the sequences of immunoglobulins of different specificities, it is of course, only necessary to prepare the heavy and light chains in separate cultures and reassemble them as desired.

For example, in order to make an anti-CEA light chain/antihepatitis heavy chain composite antibody, a suitable source for the mRNA used as a template for the light chain clone would comprise, for instance, the anti CEA producing cell line of paragraph E.1. The mRNA corresponding to heavy chain would be derived from B cells raised in response to hepatitis infection or from hybridoma in which the B cell was of this origin. It is clear that such composites can be assembled using the methods of the invention almost at will, and are limited only by available sources of mRNA suitable for use as templates for the respective chains. All other features of the process are similar to those described above.

D.5 Hybrid Antibodies

Hybrid antibodies are particularly useful as they are capable of simultaneous reaction with more than one antigen. Pairs of heavy and light chains corresponding to chains

of antibodies for different antigens, such as those set forth in paragraph D.4 are prepared in four separate cultures, thus preventing premature assembly of the tetramer. Subsequent mixing of the four separately prepared peptides then permits assembly into the desired tetramers. While random aggregation may lead to the formation of considerable undesired product, that portion of the product in which homologous light and heavy chains are bound to each other and mismatched to another pair gives the desired hybrid antibody.

D.6 Chimeric Antibodies

For construction of chimeric antibodies (wherein, for example, the variable sequences are separately derived from the constant sequences) the procedures of paragraph C.1 and D.2 are again applicable with appropriate additions and modifications. A preferred procedure is to recover desired portions of the genes encoding for parts of the heavy and light chains from suitable, differing, sources and then to religate these fragments using restriction endonucleases to reconstruct the gene coding for each chain.

For example, in a particularly preferred chimeric construction, portions of the heavy chain gene and of the light chain gene which encode the variable sequences of antibodies produced by a murine hybridoma culture are recovered and cloned from this culture and gene fragments encoding the constant regions of the heavy and light chains for human antibodies recovered and cloned from, for example, human myeloma cells. Suitable restriction enzymes may then be used to ligate the variable portions of the mouse gene to the constant regions of the human gene for each of the two chains. The chimeric chains are produced as set forth in D.1, aggregated as set forth in D.2 and used in the same manner as the non-chimeric forms. Of course, any splice point in the chains can be chosen.

D.7 Altered Antibodies

Altered antibodies present, in essence, an extension of chimeric ones. Again, the techniques of D.1 and D.2 are applicable; however, rather than splicing portions of the chain(s), suitable amino acid alterations, deletions or additions are made using available techniques such as mutagenesis (supra). For example, genes which encode antibodies having diminished complement fixation properties, or which have enhanced metal binding capacities are prepared using such techniques. The latter type may, for example, take advantage of the known gene sequence encoding metallothionein II (Karin, M., et al., *Nature*, 299: 797 (1982)). The chelating properties of this molecular fragment are useful in carrying heavy metals to tumor sites as an aid in tumor imaging (Scheinberg, D. A., et al., *Science*, 215: 19 (1982).

D.8 Univalent Antibodies

In another preferred embodiment, antibodies are formed which comprise one heavy and light chain pair coupled with the Fc region of a third (heavy) chain. These antibodies have a particularly useful property. They can, like ordinary antibodies, be used to target antigenic surfaces of tissues, such as tumors, but, unlike ordinary antibodies, they do not cause the antigenic surfaces of the target tissue to retreat and become non-receptive. Ordinary antibody use results in aggregation and subsequent inactivation, for several hours, of such surface antigens.

The method of construction of univalent antibodies is a straightforward application of the invention. The gene for heavy chain of the desired Fc region is cleaved by restriction enzymes, and only that portion coding for the desired Fc region expressed. This portion is then bound using the technique of D.2 to separately produced heavy chain the desired pairs separated from heavy/heavy and Fc/Fc combinations, and separately produced light chain added. Pre-binding of the two heavy chain portions thus diminishes the probability of formation of ordinary antibody.

D.9 Fab Protein

Similarly, it is not necessary to include the entire gene for the heavy chain portion. All of the aforementioned variations can be superimposed on a procedure for Fab protein production and the overall procedure differs only in that the portion of the heavy chain coding for the amino terminal 220 amino acids is employed in the appropriate expression vector.

E. Specific Examples of Preferred Embodiments

The invention has been described above in general terms and there follow several specific examples of embodiments which set forth details of experimental procedure in producing the desired antibodies. Example E.1 sets forth the general procedure for preparing anti CEA antibody components, i.e. for a "mammalian antibody". Example E.3 sets forth the procedure for reconstitution and thus is applicable to preparation of mammalian, composite, hybrid and chimeric immunoglobulins, and Fab proteins and univalent antibodies. Example E.4 sets forth the procedure for tailoring the heavy or light chain so that the variable and constant regions may be derived from different sources. Example E.5 sets forth the method of obtaining a shortened heavy chain genome which permits the production of the Fab regions and, in an analogous manner, Fc region.

The examples set forth below are included for illustrative purposes and do not limit the scope of the invention.

E.1 Construction of Expression Vectors for Murine anti-CEA Antibody Chains and Peptide Synthesis

Carcinoembryonic antigen (CEA) is associated with the surface of certain tumor cells of human origin (Gold, P., et al., *J. Exp. Med.*, 122: 467 (1965)). Antibodies which bind to CEA (anti-CEA antibodies) are useful in early detection of these tumors (Van Nagell, T. R., et al., *Cancer Res.* 40: 502 (1980)), and have the potential for use in treatment of those human tumors which appear to support CEA at their surfaces. A mouse hybridoma cell line which secretes anti-CEA antibodies of the IgG_1 class, CEA.66-E3, has been prepared as described by Wagener, C, et al., *J. Immunol.* (in press) which is incorporated herein by reference, and was used as mRNA source. The production of anti CEA antibodies by this cell line was determined. The N-terminal sequences of the antibodies produced by these cells was compared with those of monoclonal anti CEA as follows. Purified IgG was treated with PCAse (Podell, D. N., et al., BBRC 81: 176 (1978)), and then dissociated in 6M guanidine hydrochloride, 10 mM 2-mercaptoethanol (1.0 mg of immunoglobulin, 5 min, 100° C. water bath). The dissociated chains were separated on a Waters Associates alkyl phenyl column using a linear gradient from 100 percent A (0.1 percent TFA-water) to 90 percent B (TFA/H_2O/MeCN 0.1/9.9/90) at a flow rate of 0.8 ml/min. Three major peaks were eluted and analyzed on SDS gels by silver staining. The first two peaks were pure light chain (MW 25,000 daltons), the third peak showed a (7:3) mixture of heavy and light chain. 1.2 nmoles of light chain were sequenced by the method of Shively, J. E., *Methods in Enzymology*, 79: 31 (1981), with an NH_2-terminal yield of 0.4 nmoles. A mixture of heavy and light chains (3 nmoles) was also sequenced, and sequence of light chain was deducted from the double sequence to yield the sequence of the heavy chain.

In the description which follows, isolation and expression of the genes for the heavy and light chains for anti CEA antibody produced by CEA.66-E3 are described. As the constant regions of these chains belong to the *gamma* and kappa families, respectively, "light chain" and "kappa

chain", and "heavy chain" and "gamma chain", respectively, are used interchangeably below.

E.1.1 Isolation of Messenger RNA for Anti CEA Light and Heavy (Kappa and Gamma) Chains

Total RNA from CEA.66-E3 cells was extracted essentially as reported by Lynch et al, *Virology*, 98: 251 (1979). Cells were pelleted by centrifugation and approximately 1 g portions of pellet resuspended in 10 ml of 10 mM NaCl, 10 mM Tris HCl (pH 7.4), 1.5 mM $MgCl_2$. The resuspended cells were lysed by addition of non-ionic detergent NP-40 to a final concentration of 1 percent, and nuclei removed by centrifugation. After addition of SDS (pH 7.4) to 1 percent final concentration, the supernatant was extracted twice with 3 ml portions of phenol (redistilled)/chloroform: isoamyl alcohol 25:1 at 4° C. The aqueous phase was made 0.2 M in NaCl and total RNA was precipitated by addition of two volumes of 100 percent ethanol and overnight storage at −20° C. After centrifugation, polyA mRNA was purified from total RNA by oligo-dT cellulose chromatography as described by Aviv and Leder, *Proc. Nat'l Acad. Sci. (USA)*, 69: 1408 (19672), 142 μg of polyA mRNA was obtained from 1 g cells.

E.1.2 Preparation of *E. coli* Colony Library Containing Plasmids with Heavy and Light DNA Sequence Inserts

5 μg of the unfractionated polyA mRNA prepared in paragraph E.1.1 was used as template for oligo-dT primed preparation of double-stranded (ds) cDNA by standard procedures as described by Goeddel et al., *Nature* 281: 544 (1979) and Wickens et al., *J. Biol. Chem.* 253: 2483 (1978) incorporated herein by reference. The cDNA was size fractionated by 6 percent polyacrylamide gel electrophoresis and 124 ng of ds cDNA greater than 600 base pairs in length was recovered by electroelution. A 20 ng portion of ds cDNA was extended with deoxy C residues using terminal deoxynucleotidyl transferase as described in Chang et al., *Nature* 275: 617 (1978) incorporated herein by reference, and annealed with 200 ng of the plasmid pBR322 (Bolivar et al., *Gene* 2: 95 (1977)) which had been cleaved with Pst I and tailed with deoxy G. Each annealed mixture was then transformed into *E. coli* K12 strain 294 (ATCC No. 31446). Approximately 8500 ampicillin sensitive, tetracycline resistant transformants were obtained.

E.1.3 Preparation of Synthetic Probes

The 14mer, 5' GGTGGGAAGATGGA 3' complementary to the coding sequence of constant region for mouse MOPC21 kappa chain which begins 25 basepairs 3' of the variable region DNA sequence was used as kappa chain probe. A 15 mer, 5' GACCAGGCATCCCAG 3', complementary to a coding sequence located 72 basepairs 3' of the variable region DNA sequence for mouse MOPC21 gamma chain was used to probe gamma chain gene.

Both probes were synthesized by the phosphotriester method described in German Offenlegungschrift 2644432, incorporated herein by reference, and made radioactive by kinasing as follows: 250 ng of deoxyoligonucleotide were combined in 25 μl of 60 mM Tris HCl (pH 8), 10 mM $MgCl_2$, 15 mM beta-mercaptoethanol, and 100 μCi (γ-^{32}P) ATP (Amersham, 5000 Ci/mMole). 5 units of T4 polynucleotide kinase were added and the reduction was allowed to proceed at 37° C. for 30 minutes and terminated by addition of EDTA to 20 mM.

E.1.4 Screening of Colony Library for Kappa or Gamma Chain Sequences

~2000 colonies prepared as described in paragraph E.1.2 were individually inoculated into wells of microtitre dishes containing LB (Miller, Experiments in Molecular Genetics, p. 431–3, Cold Spring Harbor, Lab., Cold Spring Harbor, N.Y. (1972))+5 μg/ml tetracycline and stored at −20° C. after addition of DMSO to 7 percent. Individual colonies from this library were transferred to duplicate sets of Schleicher and Schuell BA85/20 nitrocellulose filters and grown on agar plates containing LB+5 μg/ml tetracycline. After ~10 hours growth at 37° C. the colony filters were transferred to agar plates containing LB+5 μg/ml tetracycline and 12.5 μg/ml chloramphenicol and reincubated overnight at 37° C. The DNA from each colony was then denatured and fixed to the filter by a modification of the Grunstein-Hogness procedure as described in Grunstein et al., *Proc. Natl. Acad. Sci. (USA)* 72: 3961 (1975), incorporated herein by reference. Each filter was floated for 3 minutes on 0.5 N NaOH, 1.5 M NaCl to lyse the colonies and denature the DNA then neutralized by floating for 15 minutes on 3 M NaCl, 0.5 M Tris HCl (pH 7.5). The filters were then floated for an additional 15 minutes on 2XSSC, and subsequently baked for 2 hours in an 80° C. vacuum oven. The filters were prehybridized for ~2 hours at room temperature in 0.9 M NaCl, 1X Denhardts, 100 mM Tris HCl (pH 7.5), 5 mM Na-EDTA, 1 mM ATP, 1 M sodium phosphate (dibasic), 1 mM sodium pyrophosphate, 0.5 percent NP-40, and 200 μg/ml *E. coli* t-RNA, and hybridized in the same solution overnight, essentially as described by Wallace et al. *Nucleic Acids Research* 9: 879 (1981) using ~40×10^6 cpm of either the kinased kappa or gamma probe described above.

After extensive washing at 37° C. in 6X SSC, 0.1 percent SDS, the filters were exposed to Kodak XR-5 X-ray film with DuPont Lightning-Plus intensifying screens for 16–24 hours at −80° C. Approximately 20 colonies which hybridized with kappa chain probe and 20 which hybridized with gamma chain probe were characterized.

E.1.5 Characterization of Colonies which Hybridize to Kappa DNA Sequence Probe

Plasmid DNAs isolated from several different transformants which hybridized to kappa chain probe were cleaved with Pst I and fractionated by polyacrylamide gel electrophoresis (PAGE). This analysis demonstrated that a number of plasmid DNAs contained cDNA inserts large enough to encode full length kappa chain. The complete nucleotide sequence of the cDNA insert of one of these plasmids was determined by the dideoxynucleotide chain termination method as described by Smith, *Methods Enzymol.* 65, 560 (1980) incorporated herein by reference after subcloning restriction endonuclease cleavage fragments into M13 vectors (Messing et al., *Nucleic Acids Research* 9: 309 (1981). FIG. 2 shows the nucleotide sequence of the cDNA insert of pK17G4 and FIG. 3 shows the gene sequence with the corresponding amino acid sequence. Thus, the entire coding region of mouse anti-CEA kappa chain was isolated on this one large DNA fragment. The amino acid sequence of kappa chain, deduced from the nucleotide sequence of the pK17G4 cDNA insert, corresponds perfectly with the first 23 N-terminal amino acids of mature mouse anti-CEA kappa chain as determined by amino acid sequence analysis of purified mouse anti-CEA kappa chain. The coding region of pK17G4 contains 27 basepairs or 9 amino acids of the presequence and 642 basepairs or 214 amino acids of the mature protein. The mature unglycosylated protein (MW 24,553) has a variable region of 119 amino acids, including the J1 joining region of 12 amino acids, and a constant region of 107 amino acids. After the stop codon behind amino acids 215 begins 212 basepairs of 3' untranslated sequence up to the polyA addition. The kappa chain probe used to identify pK17G4 hybridizes to nucleotides 374–388 (FIG. 2).

E.1.6 Characterization of Colonies which Hybridize to Gamma 1 DNA Probe

Plasmid DNA isolated from several transformants positive for hybridization with the heavy chain gamma 1 probe was subjected to Pst I restriction endonuclease analysis as described in E.1.5. Plasmid DNAs demonstrating the largest cDNA insert fragments were selected for further study. Nucleotide sequence coding for mouse heavy (gamma-1) chain, shows an NcoI restriction endonuclease cleavage site near the junction between variable and constant region. Selected plasmid DNAs were digested with both PstI and NcoI and sized on polyacrylamide. This analysis allowed identification of a number of plasmid DNAs that contain NcoI restriction endonuclease sites, although none that demonstrate cDNA insert fragments large enough to encode the entire coding region of mouse anti-CEA heavy chain.

In one plasmid isolated, p γ298 the cDNA insert of about 1300 bp contains sequence information for the 5' untranslated region, the signal sequence and the N-terminal portion of heavy chain. Because pγ298 did not encode the C-terminal sequence for mouse anti-CEA gamma 1 chain, plasmid DNA was isolated from other colonies and screened with PstI and NcoI. The C-terminal region of the cDNA insert of pγ11 was sequence and shown to contain the stop codon, 3' untranslated sequence and that portion of the coding sequence missing from p γ298.

FIG. 4 presents the entire nucleotide sequence of mouse anti-CEA heavy chain (as determined by the dideoxynucleotide chain termination method of Smith, *Methods Enzymol.*, 65: 560 (1980)) and FIG. 5 includes the translated sequence.

The amino acid sequence of gamma 1 (heavy chain) deduced from the nucleotide sequence of the pγ298 cDNA insert corresponds perfectly to the first 23 N-terminal amino acids of mature mouse anti-CEA gamma 1 chain as determined by amino acid sequence analysis of purified mouse anti-CEA gamma-1 chain. The coding region consists of 57 basepairs or 19 amino acids of presequences and 1346 basepairs or 447 amino acids of mature protein. The mature unglycosolated protein (MW 52,258) has a variable region of 135 amino acids, including a D region of 12 amino acids, and a J4 joining region of 13 amino acids. The constant region is 324 amino acids. After the stop codon behind amino acid 447 begins 96 bp of 3' untranslated sequences up to the polyA addition. The probe used to identify pγ298 and pγ11 hybridized to nucleotides 528–542 (FIG. 4).

E.1.7 Construction of a Plasmid for Direct Expression of Mouse Mature Anti-CEA Kappa Chain Gene, pKCEAtrp207-1*

FIG. 6 illustrated the construction of pKCEAtrp207-1*

First, an intermediate plasmid pHGH207-1*, having a single trp promoter, was prepared as follows:

The plasmid pHGH 207 (described in U.S. patent application Ser. No. 307,473, filed Oct. 1, 1981 (EPO Publn. No. 0036776)) has a double lac promoter followed by the trp promoter, flanked by EcoR I sites and was used to prepare pHGH207-1. pHGH207 was digested with BamH I, followed by partial digestion with EcoR I. The largest fragment, which contains the entire trp promoter, was isolated and ligated to the largest EcoR I- BamH I fragment from pBR322, and the ligation mixture used to transform *E. coli* 294. Tet^R Amp^R colonies were isolated, and most of them contained pHGH207-1. pHGH207-1* which lacks the EcoR1 site between the amp^R gene and the trp promoter, was obtained by partial digestion of pHGH207-1 with EcoR I, filling in the ends with Klenow and dNTPs, and religation.

5 μg of pHGH207-1* was digested with EcoRI, and the ends extended to blunt ends using 12 units of DNA Polymerase I in a 50 μl reaction containing 60 mM NaCl, 7 mM $MgCl_2$, 7 mM Tris HCl (pH 7.4) and 1 mM in each dNTP at 37° C. for 1 hour, followed by extraction with phenol/$CHCl_3$ and precipitation with ethanol. The precipitated DNA was digested with BamH I, and the large vector fragment (fragment 1) purified using 5 percent polyacrylamide gel electrophoresis, electroelution, phenol/$CHCl_3$ extraction and ethanol precipitation.

The DNA was resuspended in 50 μl of 10 mM Tris pH 8, 1 mM EDTA and treated with 500 units Bacterial Alkaline Phosphatase (BAP) for 30' at 65° followed by phenol/$CHCl_3$ extraction and ethanol precipitation.

A DNA fragment containing part of the light chain sequence was prepared as follows: 7 μg of pH17G4 DNA was digested with Pst I and the kappa chain containing cDNA insert was isolated by 6 percent gel electrophoresis, and electroelution. After phenol/$CHCl_3$ extraction, ethanol precipitation and resuspension in water, this fragment was digested with Ava II. The 333 bp Pst I-Ava II DNA fragment was isolated and purified from a 6 percent polyacrylamide gel.

A 15 nucleotide DNA primer was synthesized by the phosphotriester method G. O. 2,644,432 (supra) and has the following sequence:

```
Met Asp Ile Val Met
5' ATG GAC ATT GTT ATG 3'
```

The 5' methionine serves as the initiation codon. 500 ng of this primer was phosphorylated at the 5' end with 10 units T4 DNA kinase in 20 μl reaction containing 0.5 mM ATP. ~200 ng of the Pst I-Ava II DNA fragment was mixed with the 20 μl of the phosphorylated primer, heated to 95° C. for 3 minutes and quick frozen in a dry-ice ethanol bath. The denatured DNA solution was made 60 mM NaCl, 7 mM $MgCl_2$, 7 mM Tris HCl (pH 7.4), 12 mM in each dNTP and 12 units DNA Polymerase I-Large Fragment was added. After 2 hours incubation at 37° C. this primer repair reaction was phenol/$CHCl_3$ extracted, ethanol precipitated, and digested to completion with Sau 3A. The reaction mixture was then electrophoresed on a 6 percent polyacrylamide gel and ~50 ng of the 182 basepair amino-terminal blunt-end to Sau 3A fragment (fragment 2) was obtained after electroelution.

100 ng of fragment 1 (supra) and 50 ng of fragment 2 were combined in 20 μl of 20 mM Tris HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM DTT, 2.5 mM ATP and 1 unit of T4 DNA ligase. After overnight ligation at 14° C. the reaction was transformed into *E. coli* K12 strain 294. Restriction endonuclease digestion of plasmid DNA from a number of ampicillin resistant transformants indicated the proper construction and DNA sequence analysis proved the desired nucleotide sequence through the initiation codon of this new plasmid, pKCEAint1 (FIG. 6).

The remainder of the coding sequence of the kappa light chain gene was prepared as follows:

The Pst I cDNA insert fragment from 7 μg of K17G4 DNA was partially digested with Ava II and the Ava II cohesive ends were extended to blunt ends in a DNA Polymerase I large fragment reaction. Following 6 percent polyacrylamide gel electrophoreais the 686 basepair Pst I to blunt ended Ava II DNA fragment was isolated, purified and subjected to Hpa II restriction endonuclease digestion. The 497 basepair Hpa II to blunt ended Ava II DNA fragment (fragment 3) was isolated and purified after gel electrophoresis.

10 μg of pKCEAint1 DNA was digested with Ava I, extended with DNA polymerase I large fragment, and

digested with Xba I. Both the large blunt ended Ava I to Xba I vector fragment and the small blunt ended Ava I to Xba I fragment were isolated and purified from a 6 percent polyacrylamide gel after electrophoresis. The large vector fragment (fragment 4) was treated with Bacterial Alkaline Phosphatase (BAP), and the small fragment was digested with Hpa II, electrophoresed on a 6 percent polyacrylamide and the 169 basepair Xba I-Hpa II DNA fragment (fragment 5) was purified. ~75 ng of fragment 4, ~50 ng of fragment 3 and ~50 ng of fragment 5 were combined in a T4 DNA ligase reaction and incubated overnight at 14°, and the reaction mixture transformed into *E. coli* K12 strain 294. Plasmid DNA from six ampicillin resistant transformants were analyzed by restriction endonuclease digestion. One plasmid DNA demonstrated the proper construction and was designated pKCEAInt2.

Final construction was effected by ligating the K-CEA fragment, including the trp promoter from pKCEAInt2 into pBR322(XAP). (pBR322(XAP) is prepared as described in U.S. application Ser. No. 452,227, filed Dec. 22, 1982; from pBR322 by deletion of the AvaI-PvuII fragment followed by ligation.)

The K-CEA fragment was prepared by treating pKCEA*Int2* with Ava I, blunt ending with DNA polymerase I (Klenow fragment) in the presence of DNTPs, digestion with Pst I and isolation of the desired fragment by gel electrophoresis and electroelution.

The large vector fragment from pBR322(XAP) was prepared by successive treatment with EcoR I, blunt ending with polymerase, and redigestion with Pst I, followed by isolation of the large vector fragment by electrophoresis and electroelution.

The K-CEA and large vector fragments as prepared in the preceding paragraphs were ligated with T4 DNA ligase, and the ligation mixture transformed into *E. coli* as above. Plasmid DNA from several ampicillin resistant transformants were selected for analysis, and one plasmid DNA demonstrated the proper construction, and was designated pKCEAtrp207-1*.

E.1.8 Construction of a Plasmid Vector for Direct Expression of Mouse Mature Anti-CEA Heavy (Gamma 1) Chain Gene, pγCEAtrp207-1*

FIG. 7 illustrates the construction of pγCEA*trp*207-1*. This plasmid was constructed in two parts beginning with construction of the C-terminal region of the gamma 1 gene.

5 μg of plasmid pHGH207-1* was digested with Ava I, extended to blunt ends with DNA polymerase I large fragment (Klenow fragment), extracted with phenol/$CHCl_3$, and ethanol precipitated. The DNA was digested with BamH I treated with BAP and the large fragment (fragment A) was purified by 6 percent polyacrylamide gel electrophoresis and electroelution.

~5 μg of pγ11 was digested with Pst I and the gamma chain cDNA insert fragment containing the C-terminal portion of the gene was purified, digested with Ava II followed by extension of the Ava II cohesive ends with Klenow, followed by Taq I digestion. The 375 basepair blunt ended Ava II to Taq I fragment (fragment B) was isolated and purified by gel electrophoresis and electroelution.

9 μg of pγ298 was digested with Taq I and BamH I for isolation of the 496 basepair fragment (fragment C).

Approximately equimolar amounts of fragments A, B, and C were ligated overnight at 14° in 20 μl reaction mixture, then transformed into *E. coli* strain 294. The plasmid DNA from six ampicillin resistant transformants was committed to restriction endonuclease analysis and one plasmid DNA, named pγCEAInt, demonstrated the correct construction of the C-terminal portion of gamma 1 (FIG. 5).

To obtain the N-terminal sequences, 30 μg of pγ298 was digested with Pst I and the 628 basepair DNA fragment encoding the N-terminal region of mouse anti-CEA gamma chain was isolated and purified. This fragment was further digested with Alu I and Rsa I for isolation of the 280 basepair fragment. A 15 nucleotide DNA primer

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met glu val met leu
5' ATG GAA GTG ATG CTG 3'
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was synthesized by the phosphotriester method (supra).

The 5' methionine serves as the initiation codon. 500 ng of this synthetic oligomer primer was phosphorylated at the 5' end in a reaction with 10 units T4 DNA kinase containing 0.5 mM ATP in 20 μl reaction mixture. ~500 ng of the 280 basepair Alu I-Rsa I DNA fragment was mixed with the phosphorylated primer. The mixture was heat denatured for 3 minutes at 95° and quenched in dry-ice ethanol. The denatured DNA solution was made 60 mM NaCl, 7 mM $MgCl_2$, 7 mM Tris HCl (pH 7.4), 12 mM in each dNTP and 12 units DNA Polymerase I-Large Fragment was added. After 2 hours incubation at 37° C., this primer repair reaction was phenol/$CHCl_3$ extracted, ethanol precipitated, and digested to completion with HpaII. ~50 ng of the expected 125 basepair blunt-end to Hpa II DNA fragment (fragment D) was purified from the gel.

A second aliquot of pγ298 DNA was digested with Pst I, the 628 basepair DNA fragment purified by polyacrylamide gel electrophoresis, and further digested with BamH I and Hpa II. The resulting 380 basepair fragment (fragment E) was purified by gel electrophoresis.

~5 μg of pγCEAIntI was digested with EcoR I, the cohesive ends were made flush with DNA polymerase I (Klenow), further digested with BamH I, treated with BAP and electrophoresed on a 6 percent polyacrylamide gel. The large vector fragment (fragment F) was isolated and purified.

In a three fragment ligation, 50 ng fragment D, 100 ng fragment E, and 100 ng fragment F were ligated overnight at 4° in a 20 μl reaction mixture and used to transform *E. coli* K12 strain 294. The plasmid DNAs from 12 ampicillin resistant transformants were analyzed for the correct construction and the nucleotide sequence surrounding the initiation codon was verified to be correct for the plasmid named pγCEAInt2.

The expression plasmid, pγCEAtrp207-1* used for expression of the heavy chain gene is prepared by a 3-way ligation using the large vector fragment from pBR322(XAP) (supra) and two fragments prepared from pγCEAInt2.

pBR322(XAP) was treated as above by digestion with EcoR1, blunt ending with DNA polymerase (Klenow) in the presence of dNTPs, followed by digestion with Pst I, and isolation of the large vector fragment by gel electrophoresis. A 1543 base pair fragment from pγCEAInt2 containing trp promoter linked with the N-*terminal* coding region of the heavy chain gene was isolated by treating pγCEAInt2 with Pst I followed by BamH I, and isolation of the desired fragment using PAGE. The 869 base pair fragment containing the C-terminal coding portion of the gene was prepared by partial digestion of pγCEAInt2 with Ava I, blunt ending with Klenow, and subsequent digestion with BamH I, followed by purification of the desired fragment by gel electrophoresis.

The aforementioned three fragments were then ligated under standard conditions using T4 DNA ligase, and a ligation mixture used to transform *E. coli* strain 294. Plasmid DNAs from several tetracycline resistant transformants were analyzed; one plasmid DNA demonstrated the proper construction and was designated pγCEAtrp207-1*.

E.1.9 Production of Immunoglobulin Chains by *E. coli*

E. coli strain W3110 (ATTC No. 27325) was transformed with pyCEAtrp207-1* or pKCEAtrp207-1* using standard techniques.

To obtain double transformants, *E. coli* strain W3110 cells were transformed with a modified pKCEAtrp207-1*, pKCEA*trp207-1*Δ*, which had been modified by cleaving a Pst I-Pvu I fragment from the amp^R gene and religating. Cells transformed with pKCEAtrp207-1*Δ are thus sensitive to ampicillin but still resistant to tetracycline. Successful transformants were retransformed using pyCEAInt2 which confers resistance to ampicillin but not tetracycline. Cells containing both pKCEAtrp207-1*Δ and pyCEAInt2 thus identified by growth in a medium containing both ampicillin and tetracycline.

To confirm the production of heavy and/or light chains in the transformed cells, the cell samples were inoculated into M9 tryptophan free medium containing 10 μg/ml tetracycline, and induced with indoleacrylic acid (IAA) when the OD 550 reads 0.5. The induced cells were grown at 37° C. during various time periods and then spun down, and suspended in TE buffer containing 2 percent SDS and 0.1 M β-mercaptoethanol and boiled for 5 minutes. A 10xvolume of acetone was added and the cells kept at 22° C. for 10 minutes, then centrifuged at 12,000 rpm. The precipitate was suspended in O'Farrell SDS sample buffer (O'Farrell, P. H., *J. Biol. Chem.*, 250: 4007 (1975)); boiled 3 minutes, recentrifuged, and fractionated using SDS PAGE (10 percent), and stained with silver stain (Goldman, D. et al., *Science* 211: 1437 (1981)); or subjected to Western blot using rabbit anti-mouse IgG (Burnett, W. N., et al., *Anal. Biochem.* 112: 195 (1981)), for identification light chain and heavy chain.

Cells transformed with pyCEAtrp207-1* showed bands upon SDS PAGE corresponding to heavy chain molecular weight as developed by silver stain. Cells transformed with pKCEAtrp207-1* showed the proper molecular weight band for light chain as identified by Western blot; double transformed cells showed bands for both heavy and light chain molecular weight proteins when developed using rabbit anti-mouse IgG by Western blot. These results are shown in FIGS. 8A, 8B, and 8C.

FIG. 8A shows results developed by silver stain from cells transformed with pyCEAtrp207-1*. Lane 1 is monoclonal anti-CEA heavy chain (standard) from CEA.66-B3. Lanes 2b–5b are timed samples 2 hrs, 4 hrs, 6 hrs, and 24 hrs after IAA addition. Lanes 2a–5a are corresponding untransformed controls; Lanes 2c–5c are corresponding uninduced transformants.

FIG. 8B shows results developed by Western blot from cells transformed with pKCEAtrp207-1*. Lanes 1b–6b are extracts from induced cell immediately, 1 hr, 3.5 hrs, 5 hrs, 8 hrs, and 24 hrs after IAA addition, and 1a–6a corresponding uninduced controls. Lane 7 is an extract from a pyCEAtrp207-1* control, lanes 8, 9, and 10 are varying amounts of anti CEA-kappa chain from CEA.66-E3 cells.

FIG. 8C shows results developed by Western blot from four colonies of double transformed cells 24 hours after IAA addition (lanes 4–7). Lanes 1–3 are varying amounts of monoclonal gamma chain controls, lanes 8 and 9 are untransformed and pyCEAtrp207-1* transformed cell extracts, respectively.

In another quantitative assay, frozen, transformed *E. coli* cells grown according to E.1.10 (below) were lysed by heating in sodium dodecyl sulfate (SDS)/β-mercaptoethanol cell lysis buffer at 100°. Aliquots were loaded on an SDS polyacrylamide gel next to lanes loaded with various amounts of hybridoma anti-CEA. The gel was developed by the Western blot, Burnett (supra), using ^{125}I-labeled sheep anti-mouse IgG antibody from New England Nuclear. The results are shown in FIG. 9. The figure shows that the *E. coli* products co-migrate with the authentic hybridoma chains, indicating no detectable proteolytic degradation in *E. coli*. Heavy chain from mammalian cells is expected to be slightly heavier than *E. coli* material due to glycosylation in the former. Using the hybridoma lanes as a standard, the following estimates of heavy and light chain production were made:

	(Per gram of cells)
E. coli (W3110/pyCEAtrp207-1*)	5 mg γ
E. coli (W3110/pKCEAtrp207-1*)	1.5 mg K
E. coli (W3110/pKCEAtrp207-1*A, pyCEAInt2)	0.5 mg K, 1.0 mg γ

E.1.10 Reconstitution of Antibody from Recombinant K and Gamma Chains

In order to obtain heavy and light chain preparations for reconstitution, transformed cells were grown in larger batches, harvested and frozen. Conditions of growth of the variously transformed cells were as follows:

E. coli (W3110/pyCEAtrp207-1*) were inoculated into 500 ml LB medium containing 5 μg/ml tetracycline and grown on a rotary shaker for 8 hours. The culture was then transferred to 10 liters of fermentation medium containing yeast nutrients, salts, glucose, and 2 μg/ml tetracycline. Additional glucose was added during growth and at OD 550=20, indoleacrylic (IAA), a trp derepressor, was added to a concentration of 50 μg/ml. The cells were fed additional glucose to a final OD 550=40, achieved approximately 6 hours from the IAA addition.

E. coli (W3110) cells transformed with pKCEA trp 207-1* and double transformed (with pKCEAtrp207-1*Δ and pyCEAInt2) were grown in a manner analogous to that described above except that the OD 550 six hours after IAA addition at harvest was 25–30.

The cells were then harvested by centrifugation, and frozen.

E.2 Assay Method for Reconstituted Antibody

Anti-CEA activity was determined by ELISA as a criterion for successful reconstitution. Wells of microtiter plates (Dynatech Immulon) were saturated with CEA by incubating 100 μl of 2–5 μg CEA/ml solution in 0.1M carbonate buffer, pH 9.3 for 12 hours at room temperature. The wells were then washed 4 times with phosphate buffered saline (PBS), and then saturated with BSA by incubating 200 μl of 0.5 percent BSA in PBS for 2 hours at 37° C., followed by washing 4 times with PBS. Fifty microliters of each sample was applied to each well. A standard curve (shown in FIG. 10), was run, which consisted of 50 μl samples of 10 μg, 5 μg, 1 μg, 500 ng, 100 ng, 50 ng, 10 ng, 5 ng and 1 ng anti-CEA/ml in 0.5 percent BSA in PBS, plus 50 μl of 0.5 percent BSA in PBS alone as a blank. All of the samples were incubated in the plate for 90 minutes at 37° C.

The plates were then washed 4 times with PBS, and sheep anti-mouse IgG-alkaline phosphate (TAGO, Inc.) was applied to each well by adding 100 μl of an enzyme concentration of 24 units/ml in 0.5 percent BSA in PBS. The solution was incubated at 37° C. for 90 minutes. The plates were washed 4 times with PBS before adding the substrate, 100 μl of a 0.4 mg/ml solution of p-nitrophenylphosphate (Sigma) in ethanolamine buffered saline, pH 9.5. The substrate was incubated 90 minutes at 37° C. for color development.

The A_{450} of each well was read by the Microelisa Auto Reader (Dynatech) set to a threshold of 1.5, calibration of 1.0 and the 0.5 percent BSA in PBS (Blank) well set to 0.000. The A_{450} data was tabulated in RS-1 on the VAX system, and the standard curve data fitted to a four-parameter logistic model. The unknown samples' concentration were calculated based on the A_{450} data.

E.3 Reconstitution of Recombinant Antibody and Assay

Frozen cells prepared as described in paragraph E.1.10 were thawed in cold lysis buffer [10 mM Tris HCl, pH 7.5, 1 mM EDTA, 0.1M NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF)] and lysed by sonication. The lysate was partially clarified by centrifugation for 20 mins at 30,000 rpm. The supernatant was protected from proteolytic enzymes by an additional 1 mM PMSF, and used immediately or stored frozen at −80° C.; frozen lysates were never thawed more than once.

The S-sulfonate of *E. coli* produced anti-CEA heavy chain (γ) was prepared as follows: Recombinant *E. coli* cells transformed with pγCEAtrp207-1* which contained heavy chain as insoluble bodies, were lysed and centrifuged as above; the pellet was resuspended in the same buffer, sonicated and re-centrifuged. This pellet was washed once with buffer, then suspended in 6M guanidine HCl, 0.1M Tris HCl, pH 8, 1 mM EDTA, 20 mg/ml sodium sulfite and 10 mg/ml sodium tetrathionate and allowed to react at 25° for about 16 hrs. The reaction mixture was dialyzed against 8M urea, 0.1M Tris HCl, pH 8, and stored at 4°, to give a 3 mg/ml solution of γ-SSO_3.

650 μl of cell lysate from cells of various *E. coli* strains producing various IgG chains, was added to 500 mg urea. To this was added β-mercaptoethanol to 20 mM, Tris-HCl, pH 8.5 to 50 mM and EDTA to 1 mM, and in some experiments, γ-SSO_3 was added to 0.1 mg/ml. After standing at 25° for 30–90 mins., the reaction mixtures were dialyzed at 4° against a buffer composed of 0.1M sodium glycinate, pH 10.8, 0.5M urea, 10 mM glycine ethyl ester, 5 mM reduced glutathione, 0.1 mM oxidized glutathione. This buffer was prepared from N_2-saturated water and the dialysis was performed in a capped Wheaton bottle. After 16–48 hours, dialysis bags were transferred to 4° phosphate buffered saline containing 1 mM PMSF and dialysis continued another 16–24 hrs. Dialysates were assayed by ELISA as described in paragraph E.2 for ability to bind CEA. The results below show the values obtained by comparison with the standard curve in x ng/ml anti-CEA. Also shown are the reconstitution efficiencies calculated from the ELISA responses, minus the background (108 ng/ml) of cells producing K chain only, and from estimates of the levels of γ and K chains in the reaction mixtures.

	ng/ml anti-CEA	Percent recombination
E. coli W3110 producing IFN-αA (control)	0	—
E. coli (W3110/pKCEAtrp207-1*)	108	—
E. coli (W3110/pKCEAtrp207-1*), plus γ-SSO_3	848	0.33
E. coli (W3110/pKCEAtrp207-1*Δ, pγCEAint2)	3580	0.76
Hybridoma anti-CEA K-SSO_3 and γ-SSO_3	540	0.40

E.4 Preparation of Chimeric Antibody

FIGS. 11 and 12 show the construction of an expression vector for a chimeric heavy (gamma) chain which comprises the murine anti CEA variable region and human γ-2 constant region.

A DNA sequence encoding the human gamma-2 heavy chain is prepared as follows: the cDNA library obtained by standard techniques from a human multiple myeloma cell line is probed with 5' GGGCACTCGACACAA 3 ' to obtain the plasmid containing the cDNA insert for human gamma-2 chain (Takahashi, et al., *Cell*, 29: 671 (1982), incorporated herein by reference), and analyzed to verify its identity with the known sequence in human gamma-2 (Ellison, J., et al., *Proc. Natl. Acad. Sci. (USA)*, 79: 1984 (1982) incorporated herein by reference).

As shown in FIG. 11, two fragments are obtained from this cloned human gamma 2 plasmid (pγ2). The first fragment is formed by digestion with PvuII followed by digestion with Ava III, and purification of the smaller DNA fragment, which contains a portion of the constant region, using 6 percent PAGE. The second fragment is obtained by digesting the pγ2 with any restriction enzyme which cleaves in the 3' untranslated region of γ2, as deduced from the nucleotide sequence, filling in the Klenow and dNTPs, cleaving with Ava III, and isolating the smaller fragment using 6 percent PAGE. (The choice of a two step, two fragment composition to supply the PvuII-3' untranslated fragment provides a cleaner path to product due to the proximity of the AvaIII site to the 3 terminal end thus avoiding additional restriction sites in the gene sequence matching the 3' untranslated region site.) pγCEA207-1* is digested with EcoR 1, treated with Klenow and dNTPs to fill in the cohesive end, and digested with Pvu II, the large vector fragment containing promoter isolated by 6 percent PAGE.

The location and DNA sequence surrounding the PvuII site in the mouse gamma-1 gene are identical to the location and DNA sequence surrounding the PvuII site in the human gamma-2 gene.

The plasmid resulting from a three way ligation of the foregoing fragments, pChim1, contains, under the influence of trp promoter, the variable and part of the constant region of murine anti-CEA gamma 1 chain, and a portion of the gamma 2 human chain. pChim1 will, in fact, express a chimeric heavy chain when transformed into *E. coli*, but one wherein the change from mouse to human does not take place at the variable to constant junction.

FIG. 12 shows modification of pChim1 to construct pChim2 so that the resulting protein from expression will contain variable region from murine anti CEA antibody and constant region from the human γ-2 chain. First, a fragment is prepared from pChim1 by treating with Nco I, blunt ending with Klenow and dNTPs, cleaving with Pvu II, and isolating the large vector fragment which is almost the complete plasmid except for short segment in the constant coding region for mouse anti CEA. A second fragment is prepared from the previously described pγ2 by treating with Pvu II, followed by treating with any restriction enzyme which cleaves in the variable region, blunt ending with Klenow and dNTPs and isolating the short fragment which comprises the junction between variable and constant regions of this chain.

Ligation of the foregoing two fragments produces an intermediate plasmid which is correct except for an extraneous DNA fragment which contains a small portion of the constant region of the murine anti CEA antigen, and a small portion of the variable region of the human gamma chain. This repair can be made by excising the Xba I to Pvu II fragment and cloning into M13 phage as described by Messing et al., *Nucleic Acids Res.* 9: 309 (1981), followed by in vitro site directed deletion mutagenesis as described by Adelman, et al., *DNA*, in press (1983) which is incorporated

patent as well as certain payments from Genentech in exchange for its agreement to stipulate that the Cabilly Applicants were entitled to priority for the inventions claimed in the Boss patent. The precise terms of the settlement agreement are confidential and, despite reasonable inquiry, unknown to GSK.

17. Notably, the Boss patent would have expired by 2006. By obtaining Celltech's stipulation to priority of invention for the claimed subject matter of the Boss patent, GSK is informed and believes that Genentech sought to extend the life of patent protection for the inventions claimed in the Boss patent beyond the expiration date of the Boss patent.

18. Pursuant to the Genentech-Celltech agreement, the district court issued an order directing the PTO to vacate its determination that the Boss applicants were entitled to priority, to revoke the Boss patent, and to issue a patent to the Cabilly Applicants claiming the same subject matter as the Boss patent. The Cabilly II patent issued on December 18, 2001, and on its face is assigned to Genentech, and, by certificate of correction, is also assigned to City of Hope.

19. If the PTO Board's decision in favor of the Boss patent had not been reversed as a result of the private Genentech-Celltech agreement, the Boss patent would have expired in 2006, and the public would thereafter have been free to use the inventions claimed in the Cabilly II patent. Instead, because Genentech and Celltech agreed to request that the court reverse that result, Defendants received the Cabilly II patent, which will not expire until 2018. Consequently, due to the private Genentech-Celltech agreement, Defendants have ostensibly extended their power to exclude others from making, using, or selling the inventions claimed in the Boss and Cabilly II patent until 2018 – more than 35 years after their original 1983 patent application, and more than 12 years after the expiration of the Boss patent. The combined period of patent exclusivity secured by Defendants for the Cabilly I and Cabilly II patents, which share the same patent specification, is 29 years.

20. In 2008 alone, according to Genentech's 2009 Form 10-K filing, Defendants received $298 million in royalties on the Cabilly II patent. In short, two years after the original expiration date of the Boss patent, Genentech is receiving nearly $300 million in annual royalties on the inventions claimed in the Boss patent.

herein by reference. The Xba I-Pvu II fragment thus modified is ligated back into the intermediate plasmid to form pChim2. This plasmid then is capable of expressing in a suitable host a cleanly constructed murine variable/human constant chimeric heavy chain.

In an analogous fashion, but using mRNA templates for cDNA construction for human kappa rather than γ chain, the expression plasmid for chimeric light chain is prepared.

The foregoing two plasmids are then double transformed into *E. coli* W3110, the cells grown and the chains reconstituted as set forth in paragraph E.1–E.3 supra.

E.5 Preparation of Altered Murine Anti-CEA Antibody

E.5.1 Construction of Plasmid Vectors for Direct Expression of Altered Murine Anti-CEA Heavy Chain Gene

The cysteine residues, and the resultant disulfide bonds in the region of amino acids 216–230 in the constant region of murine anti-CEA heavy chain are suspected to be important for complement fixation (Klein, et al., *Proc. Natl. Acad. Sci.*, (*USA*), 78: 524 (1981)) but not for the antigen binding property of the resulting antibody. To decrease the probability of incorrect disulfide bond formation during reconstitution according to the process of the invention herein, the nucleotides encoding the amino acid residues 226–232 which includes codons for three cysteines, are deleted as follows:

A "deleter" deoxyoligonucelotide, 5' CTAACACCATGTCAGGGT is used to delete the relevant portions of the gene from pyCEAtrp207-1* by the procedure of Wallace, et al., *Science*, 209: 1396 (1980) or of Adelman, et al., *DNA* 2, 183 (1983). Briefly, the "deleter" deoxyoligonucelotide is annealed with denatured pyCEAtrp207-1* DNA, and primer repair synthesis carried out in vitro, followed by screening by hybridization of presumptive deletion clones with P^{32} labelled deleter sequence.

E.5.2 Production of Cysteine Deficient Altered Antibody

The plasmid prepared in E.5.1 is transformed into an *E. coli* strain previously transformed with pKCEAtrp207-1* as described above. The cells are grown, extracted for recombinant antibody chains, and the altered antibody reconstituted as described in E.1.10.

E.6 Preparation of Fab

E.6.1 Construction of a Plasmid Vector for Direct Expression of Murine Anti-CEA Gamma 1 Fab Fragment Gene pyCEAFabtrp207-1*

FIG. 13 presents the construction of pyCEAFabtrp207-1*. 5 μg of pBR322 was digested with Hind III, the cohesive ends made flush by treating with Klenow and dNTPs; digested with Pst I, and treated with BAP. The large vector fragment, fragment I, was recovered using 6 percent PAGE followed by electroelution.

5 μg of pyCEAtrp207-1* was digested with both BamH I and Pst I and the ~1570 bp DNA fragment (fragment II) containing the trp promoter and the gene sequence encoding the variable region continuing into constant region and further into the anti-CEA gamma 1 chain hinge region, was isolated and purified after electrophoresis.

Expression of the anti-CEA gamma 1 chain Fab fragment rather than complete heavy chain requires that a termination codon be constructed at the appropriate location in the gene. For this, the 260 bp Nco I-Nde I DNA fragment from 20 μg of the py298 was isolated and purified. A 13 nucleotide DNA primer, the complement of which encodes the last 3 C-terminal amino acids of the Fab gene and 2 bases of the 3 needed for the stop codon, was synthesized by the phosphotriester method (supra). The probe hybridizes to nucleotides 754 to 767 (FIG. 4) which has the following sequence:

```
    AspCysGlyStop
5' GGGATGTGGTTG  3'
```

The third base of the stop codon is provided by the terminal nucleotide of the filled-in Hind III site from pBR322 cleavage described above. 500 ng of this primer was used in a primer repair reaction by phosphorylation at the 5' end in a reaction with 10 units T4 DNA kinase containing 0.5 mM ATP in 20 μl, and mixing with ~200 ng of the Nco I-Nde I DNA fragment. The mixture was heat denatured for 3 minutes at 95° and quenched in dry-ice ethanol. The denatured DNA solution was made 60 mM NaCl, 7 mM $MgCl_2$, 7 mM Tris HCl (pH 7.4), 12 mM in each dNTP and 12 units DNA Polymerase I-Large Fragment was added. After 2 hours incubation at 37° C., this primer repair reaction was phenol/$CHCl_3$ extracted, ethanol precipitated, digested with BamH I and the reaction electrophoresed through a 6 percent polyacrylamide gel. ~50 ng of the 181 bp blunt end to BamH I DNA fragment, fragment III, was isolated and purified.

~100 ng of fragment I, ~100 ng each of fragments II and III were ligated overnight and transformed into *E. coli* K12 strain 294. Plasmid DNA from several tetracycline resistant transformants was analyzed for the proper construction and the nucleotide sequence through the repair blunt end filled-in Hind III junction was determined for verification of the TGA stop codon.

E.6.2 Production of Fab Protein

The plasmid prepared in E.6.1 is transformed into an *E. coli* strain previously transformed with pKCEAtrp207-1* as described above. The cells are grown, extracted for recombinant antibody chains and the Fab protein reconstituted as described in E.1.10.

What is claimed is:

1. A process for producing an immunoglobulin molecule or an immunologically functional immunoglobulin fragment comprising at least the variable domains of the immunoglobulin heavy and light chains, in a single host cell, comprising the steps of:

(i) transforming said single host cell with a first DNA sequence encoding at least the variable domain of the immunoglobulin heavy chain and a second DNA sequence encoding at least the variable domain of the immunoglobulin light chain, and

(ii) independently expressing said first DNA sequence and said second DNA sequence so that said immunoglobulin heavy and light chains are produced as separate molecules in said transformed single host cell.

2. The process according to claim 1 wherein said first and second DNA sequences are present in different vectors.

3. The process according to claim 1 wherein said first and second DNA sequences are present in a single vector.

4. A process according to claim 3 wherein the vector is a plasmid.

5. The process according to claim 4 wherein the plasmid is pBR322.

6. The process according to claim 1 wherein the host cell is a bacterium or yeast.

7. The process according to claim 6 wherein the host cell is *E. coli* or *S. cerevisiae*.

8. A process according to claim 7 wherein the host cell is *E. coli* strain X1776 (ATCC No. 31537).

9. A process according to claim 1 wherein the immunoglobulin heavy and light chains are expressed in the host cell and secreted therefrom as an immunologically functional immunoglobulin molecule or immunoglobulin fragment.

10. A process according to claim 1 wherein the immunoglobulin heavy and light chains are produced in insoluble form and are solubilized and allowed to refold in solution to form an immunologically functional immunoglobulin molecule or immunoglobulin fragment.

11. A process according to claim 1 wherein the DNA sequences code for the complete immunoglobulin heavy and light chains.

12. The process according to claim 1 wherein said first or said second DNA sequence further encodes at least one constant domain, wherein the constant domain is derived from the same source as the variable domain to which it is attached.

13. The process according to claim 1 wherein said first or said second DNA sequence further encodes at least one constant domain, wherein the constant domain is derived from a species or class different from that from which the variable domain to which it is attached is derived.

14. The process according to claim 1 wherein said first and second DNA sequences are derived from one or more monoclonal antibody producing hybridomas.

15. A vector comprising a first DNA sequence encoding at least a variable domain of an immunoglobulin heavy chain and a second DNA sequence encoding at least a variable domain of an immunoglobulin light chain wherein said first DNA sequence and said second DNA sequence are located in said vector at different insertion sites.

16. A vector according to claim 15 which is a plasmid.

17. A host cell transformed with a vector according to claim 15.

18. A transformed host cell comprising at least two vectors, at least one of said vectors comprising a DNA sequence encoding at least a variable domain of an immunoglobulin heavy chain and at least another one of said vectors comprising a DNA sequence encoding at least the variable domain of an immunoglobulin light chain.

19. The process of claim 1 wherein the host cell is a mammalian cell.

20. The transformed host cell of claim 18 wherein the host cell is a mammalian cell.

21. A method comprising

a) preparing a DNA sequence consisting essentially of DNA encoding an immunoglobulin consisting of an immunoglobulin heavy chain and light chain or Fab region, said immunoglobulin having specificity for a particular known antigen;

b) inserting the DNA sequence of step a) into a replicable expression vector operably linked to a suitable promoter;

c) transforming a prokaryotic or eukaryotic microbial host cell culture with the vector of step b);

d) culturing the host cell; and

e) recovering the immunoglobulin from the host cell culture, said immunoglobulin being capable of binding to a known antigen.

22. The method of claim 21 wherein the heavy and light chain are the heavy and light chains of anti-CEA antibody.

23. The method of claim 21 wherein the heavy chain is of the gamma family.

24. The method of claim 21 wherein the light chain is of the kappa family.

25. The method of claim 21 wherein the vector contains DNA encoding both a heavy chain and a light chain.

26. The method of claim 21 wherein the host cell is *E. coli* or yeast.

27. The method of claim 26 wherein the heavy chain and light chains or Fab region are deposited within the cells as insoluble particles.

28. The method of claim 27 wherein the heavy and light chains are recovered from the particles by cell lysis followed by solubilization in denaturant.

29. The method of claim 21 wherein the heavy and light chains are secreted into the medium.

30. The method of claim 21 wherein the host cell is a gram negative bacterium and the heavy and light chains are secreted into the periplasmic space of the host cell bacterium.

31. The method of claim 21 further comprising recovering both heavy and light chain and reconstituting light chain and heavy chain to form an immunoglobulin having specific affinity for a particular known antigen.

32. The insoluble particles of heavy chain and light chains or Fab region produced by the method of claim 27.

33. A process for producing an immunoglobulin molecule or an immunologically functional immunoglobulin fragment comprising at least the variable domains of the immunoglobulin heavy and light chains, in a single host cell, comprising:

independently expressing a first DNA sequence encoding at least the variable domain of the immunoglobulin heavy chain and a second DNA sequence encoding at least the variable domain of the immunoglobulin light chain so that said immunoglobulin heavy and light chains are produced as separate molecules in said single host cell transformed with said first and second DNA sequences.

34. The process of claim 9, further comprising the step of attaching the immunoglobulin molecule or immunoglobulin fragment to a label or drug.

35. The process of claim 10, further comprising the step of attaching the immunoglobulin molecule or immunoglobulin fragment to a label or drug.

36. The process of claim 33, further comprising the step of attaching the immunoglobulin molecule or immunoglobulin fragment to a label or drug.

* * * * *

EXHIBIT B

US006331415C1

(12) EX PARTE REEXAMINATION CERTIFICATE (6829th)

United States Patent

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(54) **METHODS OF PRODUCING IMMUNOGLOBULINS, VECTORS AND TRANSFORMED HOST CELLS FOR USE THEREIN**

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(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

The invention relates to processes for producing an immunoglobulin or an immunologically functional immunoglobulin fragment containing at least the variable domains of the *immunoglobulin* heavy and light chains. The processes can use one or more vectors which produce both the heavy and light chains or fragments thereof in a single cell. The invention also relates to the vectors used to produce the immunoglobulin or fragment, and to cells transformed with the vectors.

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Patent Reexamination

21. Two separate requests to re-examine the Cabilly II patent were submitted to the PTO in 2005. The PTO originally concluded that the prior art submitted by the requestors raised substantial new questions of patentability with respect to each of the claims of the Cabilly II patent and commenced separate reexamination proceedings on July 7, 2005 and January 23, 2006. *See* Decision Granting *Ex Parte* Reexamination, Reexamination Control No. 90/007,542 (July 7, 2005); Decision Granting *Ex Parte* Reexamination, Reexamination Control No. 90/007,859 (January 23, 2006). The separate reexamination proceedings were merged on June 6, 2006.

22. In an Advisory Action on July 19, 2008, the PTO maintained its final rejection of the claims in the Cabilly II patent as invalid for reasons including obviousness-type double patenting. *Ex Parte* Reexamination Advisory Action, Reexamination Control Nos. 90/007,859 and 90/007,542 (July 19, 2008).

23. In response to the final rejection, Defendants filed an Appeal Brief on December 9, 2008.

24. After an *Ex Parte* Examiner Interview on February 13, 2009, Genentech amended claims 21, 27, and 32 to overcome the obviousness-type double patenting rejection. *See* Supplemental Amendment Under 37 C.F.R. § 1.550(b) (2007), Reexamination Control Nos. 90/007,859 and 90/007,542 (February 13, 2009).

25. On February 23, 2009, the PTO issued a Notice of Intent to Issue a Reexamination Certificate to Genentech confirming claims 1-20 and 33-36 and allowing amended claims 21, 27, and 32. Notice of Intent to Issue *Ex Parte* Reexamination Certificate, Reexamination Control Nos. 90/007,859 and 90/007,542 (February 23, 2009). On May 19, 2009, the *Ex Parte* Reexamination Certificate issued for U.S. Patent 6,331,415 C1 with amended claims 21, 27, and 32. (Exhibit B).

Defendants' Admissions Regarding State of the Art in April 1983

26. In order to overcome the PTO's obviousness-type double patenting rejections during the reexamination, Defendants made a number of admissions in their December 2008 Appeal Brief regarding the state of the art prior to the filing of the Cabilly II patent application in April 1983.

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Transmittal of Brief for the Party Boss et al., Mar. 18, 1992 (Int. No. 102,572).
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Notice of Final Hearing for Mar. 29, 1994 (paper #54), Feb. 4, 1992 (Int. No. 102,572).
Cabilly et al. Supplemental Brief at Final Hearing, Apr. 5, 1992 (Int. No. 102,572).
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Communication form BPAI re: Filing of agreements and request to keep separate from interference file acknowledged (paper #59), Sep. 10, 1998 (Int. No. 102,572).
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Notice Declaring Interference, May 15, 2000 (Int. No. 104, 532).
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Glaxo Wellcome Inc. Notice of Related Litigation, Jul. 11, 2000 (Int. No. 104,532).
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Glaxo Wellcome Inc.'s Miscellaneous Motion 11(Suppression of Certain Deposition Exhibits and Deposition Testimony), Apr. 18, 2001 (Int. No. 104,532).
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Order Regarding Glaxo Wellcome Inc. Motions, May 2, 2001 (Int. No. 104,532).
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Submission of Transcript of Oral Hearing Held Sep. 18, 2001, Sep. 24, 2001 (Int. No. 104,532).
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Order Regarding Filing of Glaxo Supplemental Evidence, Nov. 13, 2001 (Int. No. 104,532).
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Cabilly Reply to Glaxo Opposition to Motion To Suppress, Nov. 27, 2001 (Int. No. 104,532).
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Notice of Allowability, Paper No. 21 dated Jun. 13, 1995 in U.S. Appl. No. 08/155,864 (Exhibit 1006; Int. No. 104,532).
Notice of Allowability, Paper No. 9 dated Jun. 7, 1995 in U.S. Appl. No. 08/335,400 (Exhibit 1007; Int. No. 104,532).
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According to Defendants, the state of the art prior to the April 1983 filing of the Cabilly patent application was as follows:

a. "[I]n April 1983, the biological mechanisms that controlled expression of foreign DNA and assembly of proteins were not well understood. This lack of understanding was especially true for eukaryotic genes, which were known to be far more complex than prokaryotic genes. As Dr. Harris, one of Owners' experts in this case, explained in his 1983 review paper, 'it is clear that not all the rules governing the expression of cloned genes have been elaborated and those rules that do exist are still largely empirical.'" (Appeal Brief at 20)

b. "In early April of 1983, the field of generic engineering was still developing . . . . A relatively small number of proteins had been made by recombinant DNA technology. Almost all of those were relatively simple monomeric (*i.e.*, one polypeptide chain) proteins." (Appeal Brief Appendix at B551 [Harris Decl.])

c. "As of April 1983, insulin was the only 'multimeric' protein that had been made using genetic engineering." (Appeal Brief at 21)

d. "Several experts with actual experience in the field of the invention in April 1983 explained that those references cited by the Examiner that include experimental results show a significant amount of unpredictability in achieving success in simpler experiments than what is required by the '415 patent claims." (Appeal Brief at 28)

e. "[S]uccessful production of immunoglobulins was highly dependent on the sequence of expression and levels at which the two immunoglobulin genes were expressed." (Appeal Brief at 63)

f. "[L]evels of expression of each immunoglobulin gene could affect production of the other immunoglobulin polypeptide." (Appeal Brief at 63)

g. "Such a person would have been familiar with the many complications of producing eukaryotic polypeptides in bacterial host cells known by April 1983." (Appeal Brief at 73).

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Deposition transcript of Herbert Heyneker (Oct. 28, 1997), with exhibits.

Deposition transcript of William E. Holmes (Oct. 19, 2007), with exhibits.

Deposition transcript of Arthur D. Riggs, M.D. (Nov. 16, 2007).

Deposition transcript of Ronald Burnell Wetzel (Nov. 29, 2007), with exhibits.

Deposition transcript of Ian M. Armitage (Oct. 16, 2007), with exhibits.

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EX PARTE REEXAMINATION CERTIFICATE ISSUED UNDER 35 U.S.C. 307

THE PATENT IS HEREBY AMENDED AS INDICATED BELOW.

Matter enclosed in heavy brackets [ ] appeared in the patent, but has been deleted and is no longer a part of the patent; matter printed in italics indicates additions made to the patent.

AS A RESULT OF REEXAMINATION, IT HAS BEEN DETERMINED THAT:

The patentability of claims **1–20** and **33–36** is confirmed.

Claims **21**, **27** and **32** are determined to be patentable as amended.

Claims **22–26** and **28–31**, dependent on an amended claim, are determined to be patentable.

21. A method comprising

a) preparing a *first* DNA sequence [consisting essentially of DNA] encoding an immunoglobulin [consisting of an immunoglobulin] heavy chain and *a second DNA sequence encoding an immunoglobulin* light chain [or Fab region, said immunoglobulin having specificity for a particular known antigen];

b) inserting the DNA [sequence] *sequences* of step a) into a replicable expression vector *wherein each sequence is* operably linked to a suitable promoter;

c) transforming a prokaryotic or eukaryotic microbial host cell culture with the vector of step b);

d) culturing the host cell *so that said immunoglobulin heavy and light chains are produced as separate molecules in said transformed host cell*; and

e) recovering the immunoglobulin from the host cell culture, said immunoglobulin being capable of binding to a known antigen.

27. The method of claim **26** wherein the heavy chain and light [chains or Fab region] *chain* are deposited within the cells as insoluble particles.

32. The insoluble particles of heavy chain and light chains [or Fab region] produced by the method of claim **27**.

* * * * *

h. "I believe a person of ordinary skill in the art, in early April of 1983, would have thought that successful expression of two immunoglobulin proteins in one transformed host cell would have been unpredictable and that assembly of the two proteins into an immunoglobulin tetramer would have been even more unpredictable." (Appeal Brief Appendix at B224 [McKnight Decl.])

i. "Experimental results would have been important to a person of ordinary skill in the art in April 1983 because many of the biological mechanisms that controlled expression of foreign DNA and assembly of proteins were not well understood at that time." (Appeal Brief Appendix at B376 [Second McKnight Decl.])

j. "Each of these papers shows that successful transformation and expression of even one foreign immunoglobulin gene in a lymphoid host cell could not be reasonably expected in April 1983. I do not believe these references can be read as suggesting that something even more challenging – expressing two different foreign immunoglobulin genes in one transformed cell – would have been something that could be predictably achieved at that time." (Appeal Brief Appendix at B382 [Second McKnight Decl.])

k. ". . . I disagree with the suggestion, that by early April 1983, my *PNAS* paper had made routine or predictable the task of expressing exogenous immunoglobulin light and heavy chain genes in the same cell. In later experiments, I attempted to use the techniques described in the *PNAS* paper to introduce and express single Ig genes into other lymphoid cell lines. Most of these experiments failed to produce stable transfectants. Thus, my experience was that using the same transfection and selection conditions described in the *PNAS* paper with other cell lines or other Ig genes did not routinely yield stable transformants containing even a single exogenous Ig gene." (Appeal Brief Appendix at B391 [Rice Decl.])